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Effect of simvastatin and ezetimibe on suPAR levels and outcomes

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ABSTRACT

Background and aims: Soluble urokinase plasminogen activator receptor (suPAR) is an inflammatory marker associated with cardiovascular disease. Statins lower both low-density lipoprotein (LDL)-cholesterol and C-reactive protein (CRP), resulting in improved outcomes. However, whether lipid-lowering therapy also lowers suPAR levels is unknown.
Methods: We investigated whether treatment with Simvastatin 40 mg and Ezetimibe 10 mg lowered plasma suPAR levels in 1838 patients with mild-moderate, asymptomatic aortic stenosis, included in the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study, using a pattern mixture model. A 1-year Cox analysis, adjusted for established cardiovascular risk factors, allocation to study treatment, peak aortic valve velocity and baseline suPAR, was performed to evaluate relationships between change in suPAR with all-cause mortality and the composite endpoint of major cardiovascular events (MCE) composed of ischemic cardiovascular events (ICE) and aortic valve related events (AVE).
Results: After 4.3 years of follow-up, suPAR levels had increased by 9.2% (95% confidence interval [CI]: 7.0%–11.5%) in the placebo group, but only by 4.1% (1.9%–6.2%) in the group with lipid-lowering treatment ($p<0.001$). In a multivariate 1-year analysis, 1-year suPAR was strongly associated with all-cause mortality, hazard ratio (HR) = 2.05 (1.17–3.61); MCE 1.40 (1.01–1.92); and AVE 1.42 (1.02–1.99) (all $p<0.042$) for each doubling of suPAR; but was not associated with ICE.
Conclusions: Simvastatin and Ezetimibe treatment impeded the progression of the time-related increase in plasma suPAR levels. Year-1 suPAR was associated with all-cause mortality, MCE, and AVE irrespective of baseline levels (SEAS study: NCT00092677).

1. Introduction

Soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of urokinase plasminogen activator receptor (uPAR), a membrane-linked receptor present on immunologically active cells, vascular endothelial cells and more recently, immature myeloid cells [1]. UPAR and circulating suPAR have been implicated in many of the pathways involved in atherosclerosis, including the plasminogen activating pathway, inflammation, modulation of cell adhesion, migration, and proliferation [2]. SuPAR has been associated with several cardiovascular diseases, including adverse

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outcomes in patients with mild-moderate aortic stenosis (AS) [3,4]. It has previously been suggested that suPAR could reflect the early inflammation-mediated phase of AS [4]. Though it is unclear at present whether suPAR is a pathogenic agent in cardiovascular disease, evidence suggests that suPAR may be more than a passive biomarker, and has recently been shown to be a causative factor in the development of chronic kidney disease [5–7].

Atherosclerosis is an inflammatory driven process involving complex interactions between components of the immune system, inflammatory mediators, and atherogenic lipoproteins, with accelerated atherosclerosis eventually leading to plaque rupture and ischemic events [8]. Our enhanced understanding of inflammation in atherogenesis has generated interest in targeting these inflammatory pathways to prevent cardiovascular disease. For example, the recent Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial was successful in reducing major cardiovascular events through inhibition of the cytokine interleukin-1β with Canakinumab, and further landmark trials with other anti-inflammatory treatments are underway [9,10]. Statins are known to have anti-inflammatory properties beyond their role as lipid-lowering medications, and studies have shown that statin treatment reduced cardiovascular events and mortality for individuals with high-levels of inflammation despite them having low-levels of low-density lipoprotein (LDL)-cholesterol [11,12]. However, it is not known whether statin therapy per se affects suPAR levels, or whether modulation of suPAR levels results in improved cardiovascular outcome. This is of particular interest as suPAR appears to reflect an alternative inflammatory pathway to c-reactive protein (CRP), more closely aligned with atherosclerosis, which potentially may yield new treatment options [3].

We, therefore, hypothesized that lipid-lowering treatment with Simvastatin and Ezetimibe would reduce suPAR levels; and secondly, that reduction in plasma suPAR levels would be associated with fewer cardiovascular events. We tested these two hypotheses in patients with mild-moderate, asymptomatic AS: a substudy of the SEAS study.

2. Materials and methods

2.1. Study design and patient population

The SEAS study originally evaluated the potential of lipid-lowering statin treatment to minimize aortic valve events, cardiovascular disease, and death. The baseline characteristics, design protocol and results of the main SEAS study have been published in detail [13,14]. In brief, 1873 patients with mild-moderate AS, as determined by Doppler ultrasound (aortic peak velocity $\geq$2.5 and $\leq$ 4.0 m/sec), were randomized to treatment with either Simvastatin 40 mg and Ezetimibe 10 mg or placebo over a 4.3 year period. In this substudy, serial plasma suPAR levels were measured at baseline, 1-year, 4-years and the study end-date (4.3-years) in 1838 patients (aged 28–86 years; 39% female). Of these patients, 15 were excluded due to the serum suPAR level being outside of the range of the enzyme-linked immunosorbent assay (ELISA).

All patients gave written informed consent, and the ethical committees of all participating countries (Norway, Sweden, Denmark, Finland, Germany, the United Kingdom, and Ireland) approved the study (the SEAS study is registered at http://ClinicalTrials.gov, identifier NCT00092677).

2.2. Biochemical analysis

Plasma (EDTA) suPAR was analyzed using a commercial CE/IVD approved ELISA assay (suPARnostic®, ViroGates, Copenhagen, Denmark). The assay was validated to measure suPAR levels between 0.6 and 22 ng/ml. Samples were analyzed at the same time point and in the same batch, with an inter-assay coefficient of variation of 9.9%. SuPAR has been shown to be without substantial circadian variation, and the biomarker is stable in frozen samples [15].

2.3. Efficacy outcomes

We evaluated the outcomes of all-cause mortality, and the composite endpoint of major cardiovascular events (MCE) composed of ischemic cardiovascular events (ICE - a composite of cardiovascular mortality, non-fatal myocardial infarction, hospitalization for unstable angina, incidence of coronary artery bypass grafting and primary percutaneous coronary intervention procedures and non-hemorrhagic stroke); and aortic valve events (AVE - a composite of aortic-valve replacement surgery, congestive heart failure secondary to progression of AS and cardiovascular mortality).

2.4. Statistical analysis

Data were analyzed using IBM SPSS Software version 24 (IBM Corp. Chicago, Illinois, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for longitudinal data analyses. Descriptive statistics are presented as means ± standard deviation for normally distributed variables, median and interquartile range for skewed variables, and numbers and percentages for categorical variables. For comparisons of baseline characteristics, the *t*-test and the Mann-Whitney *U* test were used to compare normally and non-normally distributed variables, respectively. Categorical variables were compared using the Chi-square test. Two-tailed *p* values of less than 0.05 were considered to be statistically significant.

Due to having a skewed distribution and a log-linear relationship with clinical outcomes, suPAR was transformed with $\log_2$ before analysis. In Cox regression analysis, this implies that the hazard ratios reflect the risk associated with doubling of the suPAR concentration on the original scale.

The effect of treatment on change in suPAR was evaluated in a pattern mixture model for longitudinal data, which implicitly imputes missing values of suPAR in survivors (but not in the dead) [16]. The pattern mixture model provides optimal and unbiased inference under the assumption that missing suPAR data is *missing at random*, as results otherwise may be biased. The model included time, treatment, the time-treatment interaction, age, and gender as covariates and assumed an unstructured covariance pattern. Model assumptions were assessed in residual plots, and initial analyses revealed no further interactions between the covariates. To evaluate for potential survivor bias in the estimate of treatment effect, the proportion of survivors was compared between the treatment groups at each follow-up (no differences were found).

We performed a further multivariable 1-year Cox regression analysis for each outcome measure in a subset of 1079 patients with available biochemical data, including the predictors: 1-year suPAR, baseline suPAR, allocation to treatment group, age, sex, hypertension, body mass index, smoking status, white blood cells, total cholesterol, estimated glomerular filtration rate (eGFR) and peak aortic valve velocity. A further analysis was performed with inclusion of $\log_2$-transformed CRP. To investigate whether suPAR had a mediation role on the association between treatment allocation and adverse outcomes, analyses were repeated with and without the inclusion of suPAR as a covariate comparing the treatment effect in the two models. The proportional hazards assumption for Cox's models was robust, as verified with plots of Martingale residuals (1000 random resamplings were compared to the model's functional form) and initial analyses revealed no interactions between suPAR and other covariates.