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Short communication

Serum lipoprotein(a) is not modified by interleukin-6 receptor antagonism or associated with inflammation in non-ST-elevation myocardial infarction

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ABSTRACT

Background: The IL-6 receptor antagonist tocilizumab has been shown to attenuate the proatherogenic lipoprotein a [Lp(a)] in rheumatoid arthritis. We evaluated if a single dose of tocilizumab reduced Lp(a) in patients with non-ST-elevation myocardial infarction (NSTEMI).
Methods: Lp(a) was assessed by immunoassay (n = 117 patients) at 7 consecutive time-points between day 1 and 3 and at 3 and 6 months follow-up.
Results: Tocilizumab did not affect Lp(a) at any time-point during the study and was not associated with cardiovascular risk factors.
Conclusions: Short-time inhibition of IL-6 with tocilizumab in patients with NSTEMI did not influence Lp(a) levels.

1. Introduction

Lipoprotein (a) [Lp(a)] is a proatherogenic and prothrombotic lipoprotein with a strong hereditary component that can transverse the endothelium and interact with the tissue matrix of the arterial wall, promoting the progression of ischemic heart disease [1, 2]. Increased circulating levels of Lp(a) are associated with premature atherosclerosis, myocardial infarction and stroke [3] and is an independent predictor of poor prognosis in patients with acute coronary syndromes (ACS) [4]. Currently there are limited therapeutic options to lower Lp(a).

It has been suggested that Lp(a) may be modulated by inflammation [1], and several studies have demonstrated a decrease in Lp(a) levels in rheumatoid arthritis (RA) treated with tocilizumab [5–8]. IL-6 is a major player in ACS [9], and we recently demonstrated that tocilizumab had beneficial effects on inflammation and troponin T (TnT) release in patients with non-ST-elevation myocardial infarction (NSTEMI) [10]. To further examine the regulation of Lp(a) we analyzed (i) the relation of Lp(a) levels to inflammatory responses (i.e., CRP and leukocyte counts), (ii) and the effect of tocilizumab on Lp(a) levels in this randomized, double-blind, placebo-controlled trial in patients with NSTEMI.

2. Methods

This study was part of a randomized, double-blind, placebo controlled trial (n = 117) designed to evaluate the effect of a single dose of the anti-IL-6R antibody tocilizumab in patients with NSTEMI (ClinicalTrials.gov, NCT01491074) as described previously [10]. The study was approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Medicines Agency, and was conducted according to the Helsinki Declaration. Written, informed consent was provided by all patients. Patients were included on the day of scheduled coronary angiography. After baseline blood samples were obtained, patients received an intravenous infusion of tocilizumab 280 mg (mean dose 3.15 ± 0.51 mg/kg) or matching placebo (NaCl 0.9%) prior to coronary angiography (see [10] for details on choice of dose). After study drug administration and coronary

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angiography, 6 blood samples were obtained during the first 3 days of hospitalization (day 1: evening; day 2: morning, afternoon, evening; day 3: morning, afternoon) and at 3 and 6 months follow-up.

Peripheral venous blood collected in endotoxin-free tubes without additives and were centrifuged at >2100 *g* for 15 min after full coagulation. Serum Lp(a) (Mercodia, Uppsala, Sweden) was analyzed by enzyme immunoassay with intra- and inter-assay coefficients of variation <10%. High-sensitivity CRP (hsCRP) and troponin T (hsTnT) was measured as described [10].

Treatment effects on Lp(a) (log-transformed) were assessed using repeated measures ANOVA using baseline levels as a covariate. Within group changes were assessed using the Wilcoxon signed-rank test. Treatment effects were also analyzed after stratifying analysis according to gender, PCI, type 2 diabetes (DM2) or dichotomized baseline Lp(a) or LDL-cholesterol levels. Correlations between variables were evaluated with the Spearman Rho correlation coefficient or partial correlation adjusting for treatment. All statistical analyses were performed as two-sided tests, with $p < 0.05$ regarded as significant. A limitation of our study was that the power calculations for the study were based on CRP as the primary end point [10], and there was no power calculation for this specific sub-study. However, based on the LPA AUC and standard deviation observed in the study, a post-hoc calculation revealed we had 80% power to detect 12.5% difference in AUC between the groups and close to a 100% power to detect a 20% difference.

3. Results

Lp(a) was analyzed in a total of 117 patients (placebo n = 59, tocilizumab n = 58). Baseline clinical characteristics of the study population and correlation with baseline Lp(a) levels are shown in Table 1. Lp(a) levels were not associated with clinical demographics or biochemical markers at baseline except a week positive correlation with BMI.

There was no effect of tocilizumab on the temporal course of Lp(a) throughout the study (Fig. 1). In both treatment groups a modest increase in Lp(a) was observed giving slightly higher levels at certain time-points at day 2 and 3 of the acute phase and a decline to baseline levels during long-term follow-up (i.e. 3 and 6 months). No treatment effect was observed when stratifying analysis according to gender, PCI, DM or dicotomized baseline Lp(a) or LDL-cholesterol levels. Adjusting for treatment modality, we found no correlation between the area under the curve during the acute phase (i.e. baseline – day3) for Lp(a) and area under the curve for total and LDL-cholesterol, neutrophils, IL-6, TnT or CRP (r between 0.00 and 0.17, $p > 0.05$).

Table 1
Baseline characteristics according to treatment group and association with Lp(a).

	Placebo (n = 59)	Tocilizumab (n = 58)	P=	Corr. R=
Age (years)	60.1 ± 9.9	59.8 ± 7.7	0.859	−0.13
Female gender	5 (8.5%)	9 (15.5%)	0.364	0.01
BMI (kg/m^2)	27.4 ± 4.4	28.8 ± 3.3	0.055	0.22*
Hypertension	17 (28.8%)	26 (44.8%)	0.109	0.03
Hypercholesterolemia	13 (22.0%)	17 (29.3%)	0.491	0.14
Diabetes type 2	10 (16.9%)	10 (17.2%)	1.0	−0.04
Smoking (previous or current)	40 (67.8%)	35 (61.4%)	0.599	0.05
Heart rate (beat/min)	66 ± 13	66 ± 10	0.770	0.03
SBP (mm Hg)	137 ± 18	140 ± 18	0.389	0.03
DBP (mm Hg)	81 ± 12	83 ± 12	0.273	0.18
PCI	47 (79.7%)	41 (70.7%)	0.367	0.07
CABG	7 (11.9%)	6 (10.3%)	1.0	0.09
Medical treatment	5 (8.5%)	11 (19.0%)	0.167	−0.16
Biochemistry				
Creatinine	76 (69,90)	76 (66,83)	0.260	0.02
Neutrophils (×10^9 cells)	4.5 (3.4,6.0)	5.1 (3.4,6.3)	0.471	0.08
Interleukin-6 (pg/mL)	2.2 (1.1,4.6)	2.7 (1.2,8.7)	0.185	−0.03
C-reactive protein (mg/L)	2.5 (1.0,8.3)	2.7 (1.5,4.7)	0.458	0.00
Troponin T (ng/L)	192 (71,571)	128 (53,749)	0.965	0.04
Total-cholesterol (mmol/L)	5.3 (4.5,5.9)	4.9 (4.2,5.7)	0.279	0.05
LDL-cholesterol (mmol/L)	3.2 (2.8,3.8)	3.0 (2.3,3.6)	0.149	0.08
Medication at baseline				
Aspirin	59 (100%)	57 (98.3%)	0.496	0.09
Clopidogrel	32 (54.2%)	32 (55.2%)	1.0	−0.04
Ticagrelor	27 (45.8%)	26 (44.8%)	1.0	0.04
Low molecular weight heparin	54 (91.5%)	51 (89.5%)	0.952	−0.05
Statin	53 (89.8%)	53 (91.4%)	1.0	0.07
Betablocker	45 (76.3%)	45 (77.6%)	1.0	−0.01

Continuous data are expressed as mean ± SD or median (25th, 75th) percentiles and were compared with unpaired parametric or non-parametric tests depending on distribution. CABG, coronary artery bypass grafting; DBP, diastolic blood pressure; PCI, percutaneous coronary intervention; SBP, systolic blood pressure.
* $p < 0.05$.

4. Discussion

Our finding of a modest increase in Lp(a) in the acute phase in NSTEMI patients supports previous studies evaluating Lp(a) following MI [11–13]. In some of these studies, the change in Lp(a) correlated with changes in acute phase proteins [13] and lipids [12] suggesting that Lp(a) could play a role as an acute-phase reactant in the repair of tissue injury [13]. In our patients, the change in Lp(a) was not associated with changes in neutrophils, IL-6, TnT, CRP or total or LDL-cholesterol and our study consequently does not support Lp(a) as an acute phase reactant or linked to tissue repair or lipids. In support, more recent studies have also noted that Lp(a) levels correlated poorly with other CV risk factors [2, 14].

Cross-sectional [6] and single arm intervention studies [5] in RA patients suggest that tocilizumab treatment lowers Lp(a) levels and larger RCTs [7, 8] confirm these observations and report a 30–40% reduction in Lp(a) during tocilizumab treatment. This discrepancy between our study and studies in RA patients may have several explanations. First, medications were different between the NSTEMI patients and the RA populations. Whereas 10% used statins in the study by McInnes IB et al. [8], 90% used statins in the present study. Moreover, whereas no patients used immunosuppressive drugs, nearly all patients used immunosuppressive drugs such as methotrexate and glucocorticosteroids in addition to tocilizumab in the RA studies. Second, while the risk of developing atherosclerosis increases progressively with increasing LDL-cholesterol levels and declines with increasing levels of HDL-cholesterol in healthy individuals, the presence of an inflammatory state leads to a decrease of lipids in patients with RA, suggesting different regulation of lipid metabolism between "pure" CAD patients and patients with chronic autoimmune and inflammatory disorders [15] and IL-6 has been identified as a key driver of this dyslipidemia [16]. Third, Lp(a) levels are in general increased in patients with autoimmune disease and in particular in RA, possibly related to anti-Lp(a)-antibodies [17]. Thus, different mechanisms may regulate Lp(a) in RA compared to CAD. Fourth, tocilizumab was given as a single dose (3.2 mg/kg) in our study vs. every 4 weeks (8 mg/kg) in the RA studies. We chose a fixed dose for simplicity during the inclusion and to examine if the effect of tocilizumab was dependent on dosage per kg bodyweight. We found, however, no relation between the effect of tocilizumab on CRP or TnT, as the two most important endpoints, and body weight [10]. Finally, in the above mentioned RCT's [7, 8], the earliest samples were obtained 4–8 weeks after tocilizumab administration. The AUC for TnT was a secondary endpoint in the original study. We performed frequent sampling during the first two days to capture fluctuations in biochemical markers during the acute phase and assure

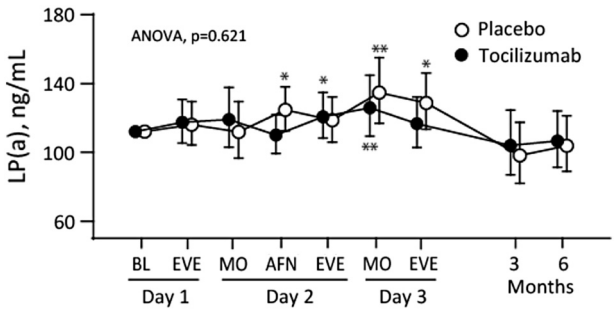

Fig. 1. Serum measures of Lp(a) in NSTEMI patients receiving placebo (n = 59, filled circles) or tocilizumab (n = 58, open circles) during hospitalization and at 3 and 6 months follow-up. Circles and bars represent geometric back-transformed estimated marginal means and 95% confidence intervals, respectively. *$p < 0.05$, **$p < 0.01$ vs. baseline (BL). EVE, evening; AFN, afternoon.

our AUC calculations would reflect peak levels of these reactants. This was particular important for TnT, which was a secondary outcome in the study. We did not include sampling at later time-points (e.g. 4 days) for simplicity/feasibility reasons. Possibly, sampling during the acute phase as in our study was too early to detect an effect on Lp(a). Thus, a higher and more frequently administered dose of tocilizumab could potentially also be related to the differences between this study and the RA studies.

Although Lp(a) levels have been suggested to be associated with inflammation in some cross-sectional studies, we found no firm evidence for this association. Some, but not all [2], studies have suggested that presence of DM2 [18] and gender [3, 19] may modify the risk associated with Lp(a) and that there may be a threshold of LDL-cholesterol or Lp (a) levels [20] that confers increase risk, but we were unable to detect any influence of these factors on treatment effects of tocilizumab in stratified analysis. Taken together, based on the neutral effect of tocilizumab on Lp(a), and lack of clinical and inflammatory correlates with Lp(a) in our NSTEMI patients, it seems that Lp(a), and potentially also other lipid parameters, may be differently regulated in CAD and chronic autoimmune and inflammatory disorders like RA.

In conclusion, our finding show that short-term inhibition of IL-6 with tocilizumab in patients with NSTEMI do not influence Lp (a) levels, and our data do not support a regulatory role of inflammation on Lp(a) levels in these patients. However, Lp(a) could be linked to inflammation in other CVD populations and should be further investigated in these.

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Conflict of interest

LG has participated in an expert meeting sponsored by F. Hoffmann-La Roche AG in 2014. The other authors declare no conflicts of interest.

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