Serum levels of lipoprotein(a) and E-selectin are reduced in rheumatoid arthritis patients treated with methotrexate or methotrexate in combination with TNF-α-inhibitor

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Abstract

Objective

To examine the effect of methotrexate (MTX) with or without tumour necrosis factor alpha (TNF-α) -inhibitors on serum lipoprotein(a) (s-Lp(a)), and to explore a possible relationship between s-Lp(a) and endothelial function (EF) in terms of serum levels of adhesion molecules and reactive hyperaemic index (RHI) in patients with rheumatoid arthritis (RA).

Methods

Serum levels of Lp(a), endothelial adhesion molecules, RHI and inflammatory markers were studied in 64 RA patients, starting with either MTX (n=34) or MTX+TNF-α-inhibitor treatment (n=30) at baseline and after 6 weeks and 6 months.

Results

Compared to baseline values, s-Lp(a) was significantly reduced after 6 weeks (p=0.001) and 6 months (p=0.001) in RA patients treated with MTX, and after 6 weeks (p=0.001) in the MTX+TNF-α-inhibitor group. A non-significant reduction was found after 6 months (p=0.102) in the MTX+TNFα-inhibitor group. Serum E-selectin (s-E-selectin) was significantly reduced in both RA treatment groups at both control points. S-Lp(a) correlated positively with s-E-selectin at baseline (p=0.004), and change in s-E-selectin correlated with the change in s-Lp(a) during follow-up ($p_{6\ weeks}$= 0.008, $p_{6\ months}$=0.009). No association was found between s-Lp(a) and the other adhesion molecules and RHI.

Conclusion

MTX or MTX combined with a TNFα-inhibitor appears to significantly reduce Lp(a). This finding indicate that s-Lp(a) might be related to systemic inflammation, or that the examined drugs might reduce s-Lp(a) by other mechanisms. Anti-inflammatory treatment might be a novel therapeutic option to decrease s-Lp(a). The associations between s-E-selectin and s-Lp(a) suggest an interaction between these factors, or a common cause.

Key words
Lp(a), endothelial function, E-selectin, rheumatoid arthritis, RHI.

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Introduction

Endothelial dysfunction, accelerated atherosclerosis and increased cardiovascular morbidity and mortality have been demonstrated in a large number of studies in rheumatoid arthritis (RA) patients during the last decades (1-5). Independent of the traditional risk factors, inflammation itself seems to have a pivotal role in provoking premature cardiovascular disease (CVD) in these patients (6). Serum lipoprotein(a) (s-Lp(a)) was discovered 45 years ago, but not until the last years, data placing s-Lp(a) as an independent predictor of CVD have become reliable (7-9). Still, the precise mechanism whereby s-Lp(a) is influencing the atherosclerotic process remains unclear. Former studies report higher levels of s-Lp(a) in RA patients compared to healthy controls (10, 11).

Lp(a) is a low density lipoprotein (LDL)-like particle produced in the liver, and consists of a lipid core surrounded by apolipoprotein B100 molecule linked through a single disulfide bond to a large glycoprotein, known as apolipoprotein(a) (12, 13). Inflammation may have an impact on this molecule. However, the results regarding this matter are thus far inconsistent (14-16). Tumour necrosis factor alpha (TNF-α) inhibitors are reported to improve the lipid profile in RA patients, but the effect on levels of s-Lp(a) has not been found significant (17, 18).

Systemic inflammation found in RA patients is characterised by activation of the vascular endothelium (19). This leads amongst others, to release of endothelial adhesion molecules; inter cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1) and E-selectin from the endothelial cell surface. Through an increased expression of these adhesion molecules on the surface of the endothelial cells, leukocytes are recruited into the arterial subendothelium, which is an important event in the atherosclerotic process (20). There is some evidence shown *in vitro* (21, 22), that Lp(a) might stimulate production of these adhesion molecules We therefore wanted to examine the possible association between s-Lp(a) and endothelial adhesion molecules, and observe how these substances are related to reactive hyperaemic index (RHI) and inflammation in RA patients. We also wanted to explore how s-Lp(a) and the leukocyte adhesion molecules are affected during treatment with methotrexate (MTX) with or without or TNFα-inhibitors.

Patients and methods

Patients

The study group comprised 64 consecutive RA patients from an ongoing prospective study called the PSARA (**PS**oriatic arthritis, **A**nkylosing spondylitis, **R**heumatoid **A**rthritis) study at Lillehammer Hospital for Rheumatic Diseases, Norway (24-26). The study is registered in Clinical Trials, NCT00902005. The control points were baseline and follow-up after 6 weeks and 6 months of treatment.

Inclusion criteria for this sub-study were: age range 18–80 years, RA according to the American College of Rheumatology (ACR) 1987 criteria (23), and clinical indication for starting treatment with MTX monotherapy or MTX in combination with adalimumab, infliximab or etanercept. The decision about treatment modality was based on conventional clinical judgement, following prevailing European/Norwegian guidelines for treatment of RA, by rheumatologists at Lillehammer Hospital for Rheumatic diseases, not involved in the study. MTX was given in doses 15-25 mg per os once a week. Etanercept was given as 50 mg subcutaneous (sc) injection once a week, adalimumab as 40 mg sc injection every other week, and infliximab as 3–5 mg/kg intravenous infusion at baseline, then following prevailing dosing schedule.

Exclusion criteria included lack of cooperability, history of or current malignancy, any recent clinically significant infection, history of tuberculosis (TB) or untreated TB, previously diagnosed immunodeficiency, pregnancy or breastfeeding, congestive heart failure, uncontrolled diabetes mellitus, recent stroke (within 3 months), systemic glucocorticosteroid (SGC) (prednislolon) dose>10mg/day during the last two weeks and use of TNF-α-inhibitor during the preceding four weeks. Of the 74 patients included, 64 completed

the study period, and were examined at baseline and at 6 weeks and 6 months follow-up. Ten patients did not complete the study period; five were excluded due to medication side effects, and five due to treatment failure. These patients had similar baseline characteristics as those who completed the study period.

The Regional Ethics Committee for Medical Research approved the study protocol, and all patients gave oral and written informed consent.

The patients underwent a physical examination by a rheumatologist. Disease Activity Scores (DAS 28) (27), complete medical history, including alcohol, coffee and tobacco use, physical activity, previous use of disease-modifying anti-rheumatic drugs (DMARDs) and current use of SGC, non-steroidal anti-inflammatory drugs (NSAIDS), statins and other drugs known to have any effect on the cardiovascular system, Body Mass Index (BMI), Health Assessment Questionnaire (HAQ) score and Visual Analogue Scales (VAS) for pain/fatigue/global were recorded (28).

Blood samples

Venous blood samples were drawn after fasting for eight hours, at baseline and at the 6 -week and 6-month follow-up. Any form of tobacco use was not allowed 12 hours before the blood samples were drawn. Serum-Lp(a) levels were analysed consecutively by particle enhanced immunoturbidimetric assay, LPALX Tina-quant® Lipoprotein (a) (Latex). The precipitate was determined turbidimetrically at 552 nm, using cobas c 501 system from Roche/Hitachi Serum E-selectin, VCAM-1 and ICAM-1 levels were determined in batch using the Quantikine colorimetric sandwich, enzyme-linked immunosorbent assay (ELISA) kits from R&D, Abington, UK, each sample tested in duplicates. Routine test standards of the hospital laboratory were used to analyse erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), leukocytes, neutrophils, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), uric acid, homocysteine, glucose and glycosylated haemoglobin (HbA1c). The laboratory assessor was blinded to the clinical data.

Endothelial function

Recently it has been demonstrated that abnormalities in pulse wave amplitude (PWA) using a novel finger plethysmograph (peripheral arterial tonometry (PAT) are significantly correlated with flow-mediated dilatation (FMD) of the brachial artery (29). FMD of the brachial artery is considered the non-invasive gold standard technique for measuring endothelial function in clinical studies. Due to this correlation and availability we chose a finger plethysmograph (EndoPAT 2000; Itamar, Caesarea, Israel) to examine the endothelial function. The EndoPAT method and the practical procedure have previously been thoroughly described (24, 26, 30, 31).

Statistics

Because s-Lp(a) had a skewed distribution, we used log-transformation to obtain an approximately symmetrical curve. To better enlighten the changes in this parameter, the real values of s-Lp(a) are also reported. The chi-square test and Fisher's exact test were used to study categorical variables, and the independent samples *t*-test, the Mann-Whitney U-test and paired *t*-test were used to identify differences in continuous variables between the two treatment groups. Except where indicated otherwise, reported values are the mean±standard deviation (SD). Linear simple regression analysis were performed with logLp(a) at baseline, and change in logLp(a) from baseline to 6 weeks and to 6 months as dependent variable in three different regression analyses.

In addition to age and gender, variables that showed a significant association ($p<0.05$) with the dependent variables were included in multivariate linear regression model. The level of statistical significance was set to 0.05. All statistical tests were two-sided, and all analyses were performed with SPSS for Windows, version 19 (SPSS Inc, Chicago, IL, USA).

Results

Baseline characteristics

Mean levels of s-Lp(a) and s-VCAM-1 were significantly lower in the RA patients starting treatment with MTX as monotherapy compared to those starting with the combination of MTX + TNF-α-inhibitor (s-Lp(a)$_{MTX}$=304 mg/L ± 352, s-Lp(a)$_{MTX+TNF-\alpha}$=575 mg/L ± 597, p=0.035, s-VCAM-1$_{MTX}$=825 ng/ml ± 217, s-VCAM-1$_{MTX+TNF-\alpha}$ = 1124 ng/ml ± 522, p=0.006). ESR, serum CRP, triglycerides, cholesterol, HDL, LDL, ICAM-1, E-selectin, uric acid, homocystein, WBC, neutrophil concentration, RHI, DAS28 calculated by ESR (DAS28(ESR)), HAQ and VAS global were similar at baseline in the two RA treatment groups. Likewise the demographic data, medication, patient history of CVD and family history of CAD (coronary artery disease) were similar, and there was no difference in number of RA patients having endothelial dysfunction at baseline between the RA groups. The RA patients in the MTX + TNF-α-inhibitor group had longer disease duration, and all of them had used a DMARD prevailing inclusion to the study (Table I). This group also had significantly higher BMI compared to the MTX group.

Improvement of cardiovascular risk markers and clinical markers during treatment

There was significant reduction of logLp(a) after 6 weeks and 6 months of treatment compared to baseline values in the MTX group. In the MTX + TNF-α-inhibitor group the reduction of logLp(a) was significant at 6 weeks, and showed a tendency of reduction after 6 months of treatment (Table II). S-E-selectin, ESR, CRP, neutrophils, DAS28(ESR) and VAS global were significantly reduced during follow-up in both RA groups (Table II). Serum levels of LDL, VCAM-1, ApoA1 and HbA1c were stable at both control points in both RA groups, whereas the serum level of HDL increased both at 6 weeks and at 6 months in the MTX group, and at 6 weeks in the MTX + TNF-α-inhibitor group, compared to baseline (Table II). Reduction of s-ICAM-1 was found after 6 weeks of treatment in both groups, however this reduction did not remain significant at the 6-month follow-up.

Using the mean serum levels of Lp(a) (without the Log transformation) at the

Table I. Baseline characteristics of RA patients starting treatment with MTX or MTX and TNF-α- inhibitor in combination.

Characteristics	MTX (n=34)	MTX + TNF-α-inhibitor (n=30)	*p*-value
Age – years	56 ± 11	58 ± 8	0.360
Women, no. (%)	25 (74)	22 (73)	1.000
Current smokers no. (%)	13 (38)	6 (20)	0.170
Any known CVD, no. (%)	3 (9)	6 (20)	0.285
Family history of CAD, no. (%)§	8 (24)	11 (37)	0.284
BMI, kg/m^2	25 ± 3	28 ± 6	0.020
RDD – years	3 ± 6	8 ± 8	0.009
Prev.DMARD, no (%) §§	4 (12)	30 (100)	0.001
RHIdys, no. (%)§§§	14 (42)	10 (33)	0.604
SGC, no (%)	8 (24)	9 (30)	0.584
Statins, no. (%)	6 (18)	6 (20)	1.000
NSAIDS, no. (%)	26 (77)	21 (70)	0.584
Ca-blocker, no. (%)	2 (6)	3 (10)	0.659
ACE-inhibitors, no. (%)	4 (12)	2 (7)	0.676
Beta blocker (%)	3 (9)	2 (7)	1.000
HT, no. (%) #	7 (21)	10 (33)	0.272
Hyperlipidaemia, no. (%)##	7 (21)	4 (13)	0.520

Except where indicated otherwise, values are the mean ± SD.
CVD: cardio-vascular disease; CAD: coronary artery disease; BMI: body mass index; RDD: rheumatic disease duration; DMARD: disease-modifying anti-rheumatic drug; RHIdys: reactive hyperaemic index <1.7; SGC: systemic glucocorticosteroids; NSAIDS: non-steroidal anti-inflammatory drugs; Ca-blocker: calcium-blocker; ACE-inhibitor: angiotensin-converting enzyme inhibitor; HT: hypertension.
§Coronary artery disease in male first-degree relatives before the age of 55 years, and/or female first-degree relatives before the age of 65 years; §§Previous use of any disease-modifying anti-rheumatic drug prevailing the study inclusion; §§§Reactive hyperaemic index <1.7; #Hypertension is defined as blood pressure ≥ 140/90 or anti-hypertensive treatment; ##Hyperlipidaemia is defined as total cholesterol > 5.5 mmol/l or lipid-lowering treatment.

control points, we found a 17% reduction of s-Lp(a) level at 6 weeks (304 mg/L to 253mg/L), and 15% reduction at 6 months (304mg/L to 259mg/L) in the MTX group, compared to baseline. In the MTX + TNF-α-inhibitor group the s-Lp(a) reduction was 15% (575 mg/L to 491 mg/L) after 6 weeks of treatment and 14% (575 mg/L to 493mg/L) after 6 months of treatment. Without the Log transformation of Lp(a) the reduction in s-Lp(a) level was significant at both control points in both treatment groups.

Variables associated with s-Lp(a) level at baseline

Independent of treatment regime, we analysed all 64 RA patients as one group in our search for variables that might be related to the s-Lp(a) level. In simple regression analysis, serum level of E-selectin was positively associated to logLp(a) at baseline (*p*=0.004 95%CI 0.004;0.021). Also age (*p*=0.035, 95%CI 0.001;0.032), use of NSAIDS (*p*=0.044, 95% CI 0.010;0.665) and CRP (*p*=0.039, 95%CI 0.000;0.018) showed positive associations to logLp(a) at baseline. In the adjusted model this association remained significant only for E-selectin (*p*=0.007 95%CI 0.003;0.019). A tendency of positive relationship was found between ESR and logLp(a) (*p*=0.095, 95%CI -0.001;0.014) and rheumatic disease duration (RDD) (*p*=0.151. 95%CI -0.005;0.033) and logLp(a) at baseline. There were no significant associations between BMI, smoking, gender, previous use of DMARD, current use of statins or GCS, known patient CVD, VCAM-1, ICAM-1, RHI, DAS28(ESR), neutrophils and logLp(a) at baseline.

Variables associated with change in serum Lp(a) level during follow-up

We continued to examine all RA patients as one group, to find variables that could be associated with the reduction in serum level of Lp(a) during follow-up. First we analysed the changes from baseline to 6 weeks (Table III). LogLp(a) was significantly positively related to s-E-selectin, CRP, ESR, age and use of NSAIDS. The positive relationship between s-E-selectin and logLp(a) remained significant in the adjusted model (*p*=0.007) (Table III).
Subsequently we examined if any of the variables were associated with the change in logLp(a) from baseline to 6 months follow-up. Also here simple regression analysis showed a significant positive association between s-E-selectin (*p*=0.001 95%CI 0.008;0.032) and age (*p*=0.035 95%CI 0.001;0.026) and logLp(a). Use of NSAIDS showed a strong tendency in relation to the reduction in logLp(a) (*p*=0.050 95%CI 0.000;0.517). In the adjusted model, where change in logLp(a) from baseline to 6 months follow-up was the dependent variable, and age, gender, use of NSAIDS and change in E-selectin from baseline to 6 months follow-up were the independent variables, the E-selectin reduction remained significantly related to the reduction in logLp(a) (*p*=0.009 95%CI 0.004;0.029). Change in ESR and CRP from baseline to 6 months follow-up showed a tendency to association with change in logLp(a) in the same period although non-significant (p_{ESR}=0.142, p_{CRP}=0.166). We found no relationships between change in RHI, DAS28(ESR), ICAM-1, VACM-1, cholesterol, HDL, LDL, triglycerides, ApoA1, treatment group, gender, BMI, smoking, known patient CVD, RDD, use of statins or GCS and change in logLp(a) from baseline to 6 months.

Discussion

LogLp(a) was significantly reduced in RA patients treated with MTX after 6 weeks and 6 months. Similar reduction was also found in the MTX+TNF-α-inhibitor group after 6 weeks, but in this group, the reduction of logLp(a) did not remain significant after 6 months. However, when we analysed the mean levels of s-Lp(a), using non-parametric tests, both treatment groups showed significant improvement in s-Lp(a) at both control points. Thus the lack of statistical significant improvement at 6 months in the MTX+TNF-α-inhibitor group might be caused by type II error, due to a relatively low sample size. It is also worth noticing that the improve-

Table II. Values of cardiovascular and inflammatory variables at baseline compared with values after 6 weeks and 6 months of treatment in the two RA groups.

	MTX					MTX + TNF-α-inhibitor				
Factors	Baseline	6 weeks	*p*-value	6 months	*p*-value	Baseline	6 weeks	*p*-value	6 months	*p*-value
LogLp(a)	2.22	2.13	0.001	2.11	0.001	2.43	2.33	0.001	2.36	0.102
E-selectin, ng/ml	44.4	40.2	0.001	39.8	0.005	51.3	42.4	0.001	43.9	0.001
VCAM-1, ng/ml	825	827	0.895	841	0.502	1124	1113	0.712	1140	0.625
ICAM-1, ng/ml	304	284	0.005	295	0.278	315	289	0.004	309	0.657
RHI	1.84	2.21	0.004	2.20	0.003	1.94	2.12	0.219	2.06	0.431
Triglycerides, mmol/L	1.27	1.09	0.011	1.22	0.542	1.32	1.31	0.940	1.32	0.902
Cholesterol, mmol/L	5.1	5.2	0.326	5.5	0.006	5.6	5.9	0.028	5.7	0.157
HDL, mmol/L	1.4	1.5	0.004	1.6	0.001	1.5	1.7	0.004	1.6	0.071
ApoA1, g/L	1.50	1.53	0.329	1.56	0.094	1.57	1.69	0.006	1.63	0.169
LDL, mmol/L	3.1	3.1	0.858	3.3	0.066	3.4	3.6	0.032	3.5	0.191
ESR, mm/ 1H	26	18	0.001	13	0.001	20	11	0.001	13	0.006
CRP, mg/L	15	7	0.001	6	0.011	14	5	0.001	5	0.009
Neutrophils, x10^9U/L	4.2	3.7	0.006	3.5	0.014	4.5	3.5	0.001	3.4	0.001
HbA1c, %	5.7	5.8	0.253	5.6	0.073	5.9	5.8	0.136	5.8	0.234
DAS28(ESR)	5.1	4.0	0.001	2.8	0.001	5.0	3.1	0.001	2.7	0.001
VAS global, mm	52	30	0.001	17	0.001	54	23	0.001	20	0.001

Except where indicated otherwise, values are the mean ± SD.
LogLp(a): Logarithmic lipoprotein(a); VCAM-1: vascular cell adhesion molecule; ICAM-1: inter cellular adhesion molecule-1; RHI: reactive hyperaemic index; HDL: high density lipoprotein; AopA1: apolipoproteinA1; LDL: low density lipoprotein; ESR: erytrhocyte sedimentation rate; CRP: C-reactive protein; HbA1c: glucosylated haemoglobin; DAS28(ESR): disease activity score calculated by ESR; VAS: visual analogue scale.

ment in s-Lp(a) seemed to happen during the first 6 weeks after initiating treatment in both groups. No further reduction was achieved between 6 weeks and 6 months, but the s-Lp(a) level remained steady.

TNF-α is a pro-inflammatory cytokine and is thought to play a crucial role in the cardiovascular burden of RA patients. It was recently reported that accumulation of oxidised LDL was induced when macrophages were stimulated with TNF-α or interleukin-6 (IL-6) (32). The same study showed that this foam cell formation was partially reversed by IL-6 blockade or TNF-α blockade. Our results may stand as a supplement to these findings, by showing improvement in endothelial cell function after initiating TNF-α-inhibitor treatment. Previous clinical studies investigating the effect of anti-rheumatic treatment on the lipid profile and s-Lp(a) levels report controversial results. Anti-TNF-α treatment in RA patients seemed to mainly affect total cholesterol, HDL and LDL, with no significant effect on s-Lp(a) (17, 18), whereas inhibition of IL-6 signalling was found to lower s-Lp(a) (33). In the study by Seriolo *et al*. the RA patients did not achieve significant reduction in the s-Lp(a) level during their 24 week treatment period (18). A possible explanation is that they used lower MTX doses (7.5-10 mg/week *vs*. 15 mg-25 mg/week). In addition this study had fewer RA patients on adalimumab than we had in the present study (n=4 *vs*. n=22). The diversity in results of the studies raises an interesting question about whether TNF-α-inhibitors might have different effect on the s-Lp(a) level in RA patients. Finally, there is always a risk of type II error in studies with small sample sizes, which in this case might explain why Seriolo *et al*. did not achieve significant reduction in s-Lp(a). In spite of the partially uncertain and controversial effects of anti-TNF-α treatment on lipids, there is a strong body of evidence that implies a beneficial role for TNF-α blockers in reducing the CVD risk and mortality in RA patients (34, 35).

In accordance with other studies (36, 37), we found a significant reduction in serum levels of the endothelial adhesion molecule, s-E-selectin during treatment with MTX in monotherapy or MTX in combination with a TNF-α-inhibitor in RA patients. However, our finding of a strong relationship between s-E-selectin and s-Lp(a) in RA patients, is to our knowledge, a novel result. One study from 1998 demonstrated a possible interaction between s-E-selectin and s-Lp(a), however this was an *in vitro* study (21). The theoretical suggestions of how s-Lp(a) is influencing the atherosclerotic process are numerous, but the exact mechanism is still unknown. Our findings demonstrated, not only a significant association between s-Lp(a) and s-E-selectin at baseline, but also their covariation during the treatment in RA patients. This is of great interest, and might bring novel insights into the understanding of s-Lp(a)'s role as a CVD risk factor. There is existing knowledge about E-selectin secretion from the endothelial cell surface, being stimulated by proinlammatory cytokines (19). In theory, high s-Lp(a) might induce expression of s-E-selectin on endothelial cells by direct or indirect effects (21), or high s-Lp(a) and s-E-selectin could both have a common cause, *e.g*. inflammation. Further studies are needed to explore these possible mechanisms.

In our study, RA patients in the MTX+TNF-α-inhibitor group had higher s-Lp(a) at baseline than the RA patients in the MTX group. Hypothetically this might be caused by longer disease duration, with a consequently longer inflammatory state in the combined group. There exists little information

Table III. Factors related to changes in Lp(a) serum level from baseline to 6 weeks follow-up in RA patients.

	logLp(a) difference					
	Unadjusted analyses			Adjusted analyses*		
Factors	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
Age	0.013	0.001;0.025	0.035	0.011	0.000;0.022	0.050
Gender	0.134	-0.127;0.395	0.309	0.054	-0.181;0.289	0.649
E-selectin diff	0.024	0.010;0.039	0.002	0.020	0.005;0.034	0.008
ESR diff	0.010	0.001;0.020	0.020	–	–	–
CRP diff	0.012	0.003;0.021	0.011	0.008	-0.002;0.018	0.118
RHI diff	-0.095	-0.251;0.062	0.232	–	–	–
DAS28 diff	0.117	-0.005;0.239	0.061	–	–	–
MTX *vs*. anti-TNF-α treatment	0.176	-0.052;0.404	0.129	–	–	–
Smoking	0.061	-0.193;0.315	0.632	–	–	–
Statins	0.031	-0.261;0.333	0.810	–	–	–
NSAIDs	0.275	0.021;0.528	0.034	0.211	-0.025;0.446	0.078

*R^2: 0.303 (Adjusted for age, gender, change in ESR, CRP, E-selectin from baseline to 6 weeks follow-up, and use of NSAIDs).
ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RHI: reactive hyperaemic index; DAS28: 28 joint count disease activity score; MTX: methotrexate; anti-TNF-α: anti-tumour necrosis factor alpha; NSAIDS: non-steroidal anti-inflammatory drugs.

regarding the influence of longstanding inflammation on s-Lp(a). However there are reports indicating that inflammation induces changes in Lp(a). For example, patients with active RA have increased levels of native, oxidised Lp(a) and Lp(a) immune complex formation (IC) (15). Another study reported increased s-Lp(a) in 93 RA patients with median disease activity (10). That study also reported a relationship between inflammation and s-Lp(a) in the patients with s-Lp(a) concentrations above 480 mg/L. Our results support this observation by a significant relationship between s-Lp(a) and CRP at baseline, and a significant relationship between change in this inflammatory marker and change in logLp(a) from baseline to 6 weeks follow-up, in the unadjusted analyses (Table III). Like former studies (11, 14) we found excess mean levels of s-Lp(a) at baseline in both RA treatment groups (>300 mg/L).

Regarding interventions to decrease levels of Lp(a), regular lipid aphaeresis in combination with lipid lowering treatment so far has shown the best results. Except for niacin, lipid modifying drugs alone, have poor reductive effect on s-Lp(a) levels (38, 39). Our results give reason to speculate whether anti-inflammatory treatment could be effective in treatment of high s-Lp(a) levels. If this is the case, we here actually present a possible new option for treating patients with high s-Lp(a). In addition, it would be interesting to examine eventual benefits of combining anti-inflammatory treatment with existing s-Lp(a) reductive treatment, like niacin. In the RA patient groups we examined, the s-Lp(a) concentration was reduced by as much as 17% during a 6 months treatment period with MTX alone or MTX in combination with a TNF-α-inhibitor. Similar reduction was also found already after 6 weeks of treatment. When we know that niacin can decrease s-Lp(a) concentration in a dose dependent fashion by approximately 20-30% (38), our result is quite promising.

To obtain more conclusive and consistent observations regarding this theme in future studies, we would suggest large RA study populations. All studies reported so far are quite small. Furthermore, it would be of great interest to compare s-Lp(a) levels in a RA group receiving MTX in combination with TNF-α-inhibitors with a control RA group, not receiving any anti-rheumatic treatment at all, in a longitudinal study design. There is also need for future studies exploring if the reduction in s-Lp(a) is an indirect consequence of the reduced inflammatory disease activity during treatment in RA patients, or if MTX or TNF-α-inhibitors have specific qualities that directly affect the s-Lp(a) level.

The present study has some limitations. The relatively small sample size, leading to type II error, could explain why there was only achieved a tendency of reduction in logLp(a) after 6 months of treatment, and not reaching statistical significance in the MTX+TNF-α-inhibitor group. The observational study design has also some disadvantages compared to a randomised controlled trial (RCT). There is always a risk for selection bias, when the selection is done by traditional clinical judgement and not by randomisation. However, the treatment groups in this study are thoroughly described, and the differences are few. An observational study also mirrors real life, and our daily clinical practice, which is an advantage. To our knowledge, this is the first clinical study that presents information regarding the seemingly strong relationship between s-Lp(a) and the endothelial adhesion molecule E-selectin in RA patients. This is also the first study directly comparing the effect of MTX and MTX in combination with a TNF-α-inhibitor on s-Lp(a) level in RA patients.

Conclusion

The initiation of MTX or MTX combined with a TNF-α-inhibitor significantly reduced s-Lp(a) after 6 weeks in RA patients. The reduction remained significant after 6 months in the MTX group, while there was a strong reduction tendency in the combination group. This might suggest that s-Lp(a) is related to systemic inflammation, or that MTX with or without TNF-α-inhibitor might reduce s-Lp(a) by other mechanisms. Thus, anti-inflammatory treatment might represent a new treatment option for patients with high s-Lp(a). Due to limited treatment options for these patients, this is of great importance. The strong associations between s-E-selectin and s-Lp(a), not only at baseline, but also as a co-variation over time, may indicate that there is an interaction between these factors, or that they have a common cause.

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