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ORIGINAL PAPER

Differential effects of inhibition of interleukin 1 and 6 on myocardial, coronary and vascular function

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Abstract

Background Anakinra, an interleukin-1 receptor antagonist and tocilizumab, an interleukin-6 receptor blocker, are used for the treatment of rheumatoid arthritis. We investigated the differential effects of anakinra and tocilizumab on myocardial and vascular function in an atherosclerosis model of patients with rheumatoid arthritis.

Methods 120 patients with rheumatoid arthritis were randomized to anakinra ($n=40$), tocilizumab ($n=40$) or prednisolone ($n=40$) for 3 months. Primary outcome measure was the change of left ventricular longitudinal strain after 3 months of treatment. Additionally, we measured coronary flow reserve, flow-mediated dilatation of the brachial artery, carotid-femoral pulse wave velocity, malondialdehyde and protein carbonyls as oxidative stress markers and C-reactive protein blood levels at baseline and post-treatment.

Results At baseline, patients among the three treatment arms had similar age, sex, disease activity score and atherosclerotic risk factors. Compared with baseline, all patients had improved longitudinal strain (− 16% vs. − 17.8%), coronary flow reserve (2.56 vs. 2.9), malondialdehyde (2.0 vs. 1.5 μM/L), protein carbonyls (0.0132 vs. 0.0115 nmol/mg), and C-reactive protein post-treatment. In all patients, the percent decrease of malondialdehyde was correlated with percent increase of longitudinal strain ($p<0.001$). Compared with tocilizumab and prednisolone, anakinra treatment resulted in a greater improvement of longitudinal strain (18.7% vs. 9.7% vs. 6%) and coronary flow reserve (29% vs. 13% vs. 1%), while pulse wave velocity and brachial blood pressure were improved only after tocilizumab treatment (11 ± 3 vs. 10.3 ± 2 m/s $p<0.05$ for all comparisons).

Conclusions Anakinra is associated with an improvement in cardiac function and tocilizumab with improvement in vascular function.

Clinical Trial Registration URL: https://http://www.clinicaltrials.gov. Unique identifier: NCT03288584.

Keywords Interleukin 1 · Interleukin 6 · Rheumatoid arthritis · Myocardial deformation · Pulse wave velocity · Coronary flow reserve

Background

Rheumatoid arthritis (RA) has been used as model to assess effects of anti-inflammatory treatment on atherosclerosis and cardiac function [1–6] because of the common mechanisms between RA and cardiovascular disease [7–9]. Anakinra, an IL-1 receptor antagonist, is used for the treatment of RA and has been shown to improve cardiac remodeling after myocardial infarction [1, 2]. Furthermore, blocking IL-6 actions by tocilizumab, a monoclonal antibody, has been proven to be therapeutically effective in RA [10].

Endothelial and LV systolic dysfunction are common in RA patients [11, 12]. Myocardial deformation imaging

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allows determination of subtle myocardial dysfunction [13–15]. We have previously shown that treatment with anakinra reduces IL-1-mediated nitro-oxidative stress, leading to an improvement of vascular function and myocardial deformation [3, 4]. Furthermore, studies have shown that endothelial and LV dysfunction may be reversible in RA patients who are treated with IL-6 inhibitors [16, 17].

In the present study, we hypothesized that IL-6 inhibition by tocilizumab would have a greater benefit on vascular and myocardial function than corticosteroid treatment and at least a similar effect to that of IL-1 inhibition by anakinra. Thus, the primary endpoint was the differential effects of tocilizumab and anakinra in comparison to the respective effects of corticosteroids on LV myocardial function as assessed by speckle tracking echocardiography. Secondary endpoint was the impact of tocilizumab and anakinra on vascular function as assessed by coronary flow reserve, flow-mediated dilatation and pulse wave velocity as well as on biomarkers of oxidative stress.

Methods

Study population and protocol

We examined 120 patients [mean age 62 (SD 12) years, 89 females] with rheumatoid arthritis (ACR/EULAR classification criteria for RA) [18] who had an inadequate response to disease modifying antirheumatic drugs (DMARDs) and corticosteroids. All patients were on methotrexate 7.5 mg once per week, leflunomide 20 mg once daily and prednisolone 5 mg once daily.

Exclusion criteria were acute coronary syndrome within the past year, familiar hyperlipidemia, insulin-dependent diabetes mellitus, history of steroid induced diabetes, chronic obstructive pulmonary disease or asthma, moderate or severe valvular heart disease, primary cardiomyopathies, renal disease (glomerular filtration rate eGFR < 60 ml/min/1.73 m^2) and malignant tumors. CAD was excluded by the absence of clinical history, angina, and reversible myocardial ischemia, as assessed by dobutamine stress echocardiography or thallium scintigraphy. None of our patients were on treatment with nonsteroidal anti-inflammatory drugs (NSAIDS) within the past year. We calculated the composite inflammatory disease activity score (DAS), (see Supplementary Appendix) [19].

Patients were randomized to receive anakinra treatment (100 mg subcutaneously once daily for 3 months ($n = 40$) after the first injection, tocilizumab treatment (160 mg subcutaneous once a week for 3 months). ($n = 40$) or were treated with an increase of their initial dose of prednisolone by 5 mg according to standard clinical practice [3] ($n = 40$) for 3 months (10 mg daily). Randomization was performed by an attending rheumatologist (PK) using a table of random numbers as reproduced from the online randomization software http://www.graphpad.com/quickcalcs/index.cfm.

Patients were examined in the outpatient clinic every 15 days to assess clinical status and CRP. To examine the patients' compliance with therapy, we asked these patients to provide the used ampoules of anakinra and tocilizumab and the used cartridges of prednisolone tablets at each visit.

The study protocol was approved by the institute's ethics committee, and written informed consents were obtained from all patients.

Echocardiography

Studies were performed using a Vivid E95 (GE Medical Systems, Horten, Norway) ultrasound system. All studies were digitally stored in a computerized station (Echopac GE, Horten, Norway) and were analyzed by two observers blinded to clinical and laboratory data. For the determination of interobserver variability, data from the first 20 patients were analyzed by the 2 readers (see also Supplementary Appendix).

2D strain and strain rate analysis

In all patients, we measured longitudinal systolic strain (LS) and systolic strain rate (LSR) from standard two-dimensional acquisitions (frame rate: 60–80/s) with the use of a dedicated software (EchoPac PC 202, GE Healthcare) [5]. Global longitudinal strain (GLS) and global longitudinal strain rate (GLSR) was calculated using the 17 LV segment model imaged from apical chamber views (4, 2 and 3 chamber view), as previously published [5]. The intra- and inter-observer variability for LV strain parameters were 8% and 9%, respectively. Normal values for GLS $\leq -20\%$ [20]. We also recorded the systolic (Sm), early diastolic (Em), and late diastolic mitral annular velocity (Am) by tissue Doppler echocardiography using standardized methodology [3] (see also Supplementary Appendix).

Coronary flow

Coronary flow velocities in the left anterior descending coronary artery were obtained with color-guided pulsed-wave Doppler from long-axis apical projections with a 7-MHz transducer [21, 22]. The maximal diastolic velocity of the overall coronary flow wave (CF-max) was measured at baseline and after adenosine infusion (140 $\mu g \times kg^{-1} \times min^{-1}$) for 3 min. CFR was calculated as the ratio of hyperemic to resting CF-max [5, 21] (see also Supplementary Appendix).

Endothelial function

Flow-mediated dilation (FMD) of the brachial artery was determined according to a previously published method and was expressed as a percentage change of the arterial diameter from the baseline vessel size [5] (see also Supplementary Appendix).

Assessment of arterial stiffness

Arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) [23–25] using arterial tonometry (Complior, Alam Medical, Vincennes, France); normal values < 10 m/s [26, 27] (see also Supplementary Appendix).

Laboratory assays

Rheumatoid factor test (positivity ≥ 15 IU/mL) was determined by nephelometry (Siemens Healthcare Diagnostics, Germany). Anti-citrullinated protein antibodies (ACPA) were determined using ELISA (Diastat, Axis-Shield Diagnostics Ltd., UK, positivity ≥ 5 U/mL) [28]. C-reactive protein (CRP) was measured by high-sensitivity particle-enhanced immunonephelometry (Dade Behring, Marburg, Germany; measurements range 0.175–1100 mg/L). Malondialdehyde and protein carbonyls were determined spectrophotometrically with a commercial kit (Oxford Biomedical Research, Rochester Hills, MI) of colorimetric assay for lipid peroxidation (measurements range 1–20 nmol/L) [29, 30].

Statistical analysis

Categorical data were compared between patients by contingency tables. Continuous variables were tested for normality using the Kolmogorov–Smirnov test. Normally distributed variables are given as mean ± standard deviation. Spearman correlation analysis was used to determine bivariate correlations. Data with a non-gaussian distribution are expressed as median (interquartile range) and were analyzed after transformation into ranks.

All analyses were intention-to-treat. ANOVA (general linear model, SPSS 22, SPSS Inc, Chicago, IL) for repeated measurements was applied (a) for measurements of the examined markers at baseline, 3 months after treatment used as a within-subject factor (b) for the effects of treatment (tocilizumab, anakinra and prednisolone), as a between-subject factors. The F and p values of the interaction between time of measurement of the examined markers and type of treatment were calculated. The F and p values of the comparison between treatments were calculated. The Greenhouse–Geisser correction was used when the sphericity assumption, as assessed by Mauchly's test, was not met. Post hoc comparisons were performed with Bonferroni correction.

Comparisons between baseline or post-treatment values of measured markers between the three treatment groups were performed using factorial ANOVA. Post hoc comparisons were performed with Bonferroni correction. Statistical significance was considered as $p < 0.05$. Baseline variables that were statistically different ($p < 0.05$) among the three study groups or were of clinical significance (sex, age, DAS, and atherosclerotic risk factors) were included in multivariate models as covariates. For post hoc analysis, Bonferroni-adjusted p values are quoted. For study sample size calculation, see Supplementary Appendix.

Results

Clinical characteristics such as age, BMI, sex, risk factors, and medications were similar among the three treatment groups (Table 1). At baseline, all treatment groups had similar values of disease activity score (DAS), biomarkers, and markers of vascular and myocardial function (Tables 1, 2, 3; $p > 0.05$ for all comparisons). DAS was similarly improved in all treatment arms at 3-month treatment (Table 3; $p = 0.9$).

Effects of treatment on vascular function, LV function and biomarkers

All patients had improved global longitudinal strain (GLS), global longitudinal systolic (GLSR S), early diastolic (GLSR E) and late diastolic (GLSR A) strain rate post-treatment ($p < 0.05$ for all comparisons).

Compared with tocilizumab and prednisolone, anakinra treatment resulted in a greater improvement of GLS (18.7% vs 9.7% vs 6%; $F = 8.7$, $p < 0.001$, Table 2; Fig. 1a), GLSR S (17.4% vs 8.5% vs 6.6%, $F = 8.6$, $p < 0.001$), and GLSR E (25.3% vs 9.9% vs 4.2%, F = 9.8, $p < 0.001$).

Anakinra treatment resulted in a greater improvement of S', Em/Am and E/Em by tissue Doppler imaging compared to tocilizumab and prednisolone treatment (Table 2, $p < 0.05$ for all comparisons).

All patients had increased coronary flow reserve (CFR) and flow-mediated dilatation (FMD) post-treatment ($p < 0.05$). However, there was a significant interaction between the type of treatment and changes in CFR and FMD post-treatment ($p < 0.05$, Table 2).

Compared with tocilizumab and prednisolone, anakinra treatment resulted greater improvement of coronary flow reserve (29% vs 13% vs 1%; $F = 9.2$, $p < 0.001$, Fig. 1b).

Anakinra and tocilizumab treatment resulted in a similar improvement of flow-mediated dilatation ($p = 0.9$) while a

Table 1 Clinical characteristics of the study population

	All patients ($n=120$)	Anakinra ($n=40$)	Tocilizumab ($n=40$)	Prednisolone ($n=40$)	p
Disease duration (years)	11 (1–26)	11 (1–27)	11 (1–26)	10 (1–25)	0.9
Disease activity score	5.5 ± 1.1	5.2 ± 0.9	5.9 ± 1.2	5.3 ± 1.1	0.9
RF (IU/ml)	14.6 ± 1.6	15.2 ± 2.2	14.7 ± 2.3	14 ± 2.2	0.8
ACPA (U/ml)	5 ± 1.9	6 ± 1.7	4.4 ± 2.1	4.8 ± 1.9	0.9
Age (years)	62 ± 12	61 ± 16	64 ± 11	62 ± 8	0.6
BMI (Kg/m^2)	29 ± 5	29 ± 7	31 ± 5	28 ± 3	0.6
Female sex, % (n)	74.1 (89)	72.5 (29)	77.5 (31)	72.5 (29)	0.9
Risk factors, % (n)					
Smoking	30.8 (37)	30 (12)	30 (12)	32.5 (13)	0.8
Hypertension	50 (60)	50 (20)	47.5 (19)	52.5 (21)	0.4
Dyslipidemia	31.6 (38)	30 (12)	32.5 (13)	32.5 (13)	0.4
Diabetes mellitus	22.5 (27)	22.5 (9)	20 (8)	25 (10)	0.6
Medication, % (n)					
ACE inhibitors	25 (30)	27.5 (11)	22.5 (9)	25 (10)	0.7
β-Blockers	12.5 (15)	12.5 (5)	12.5 (5)	12.5 (5)	0.7
Ca^{2+} channel blockers	23.3 (28)	22.5 (9)	22.5 (9)	25 (10)	0.7
Diuretics	28.3 (34)	27.5 (11)	30 (12)	27.5 (11)	0.7
Statins	11.6 (14)	10 (4)	12.5 (5)	12.5 (5)	0.5
Antidiabetics	7.5 (9)	7.5 (3)	7.5 (3)	7.5 (3)	0.9

Values for disease duration are median and interquartile range

RF rheumatoid factor, *ACPA* anti-citrullinated protein antibodies, *BMI* body mass index, *ACE* angiotensin-converting enzyme, *P p* for comparisons between the three treatment groups

Table 2 Markers of myocardial deformation, coronary microcirculation, arterial stiffness and endothelial function

	All ($n=120$)		Anakinra ($n=40$)		Tocilizumab ($n=40$)		Prednisolone ($n=40$)	
	Baseline	3 months	Baseline	3 months	Baseline	3 months	Baseline	3 months
Disease activity score	5.5 ± 1.3	3.8 ± 0.9[c]	5.2 ± 0.9	3.5 ± 0.7[c]	5.9 ± 1.2	3.9 ± 1.1[c]	5.5 ± 1.9	4.1 ± 0.8[c]
GLS (%)	− 16 ± 3.3	− 17.8 ± 3.2[b]	− 15.5 ± 4.8	− 18.4 ± 4.4[c]	− 16.1 ± 2.9	− 17.6 ± 2.5[b]	− 16.5 ± 2.8	− 17.5 ± 2.8
GLSR S (1/s)	− 0.88 ± 0.22	− 0.98 ± 0.25[b]	− 0.86 ± 0.32	− 1.01 ± 0.41[b]	− 0.88 ± 0.18	− 0.95 ± 0.17	− 0.91 ± 0.15	− 0.97 ± 0.16
GLSR E (1/s)	0.94 ± 0.31	1.06 ± 0.31[a]	0.95 ± 0.37	1.19 ± 0.38[a]	0.91 ± 0.33	1 ± 0.3[c]	0.95 ± 0.23	0.99 ± 0.26
GLSR A (1/s)	0.72 ± 0.29	0.87 ± 0.25[a]	0.7 ± 0.21	0.81 ± 0.26	0.77 ± 0.43	1.01 ± 0.21[b]	0.68 ± 0.24	0.78 ± 0.27
CFR	2.56 ± 0.6	2.9 ± 0.7[a]	2.39 ± 0.6	3.08 ± 0.5[c]	2.73 ± 0.8	3.06 ± 1[a]	2.58 ± 0.6	2.61 ± 0.6
Sm (cm/s)	8.6 ± 1.8	9.1 ± 2.2	8.4 ± 2.1	9.4 ± 2.4[b]	8.9 ± 1.6	9.5 ± 2.1	8.5 ± 1.6	8.4 ± 2.1
Em/Am	0.81 ± 0.4	0.9 ± 0.4	0.83 ± 0.4	0.99 ± 0.4[a]	0.80 ± 0.4	0.86 ± 0.4	0.81 ± 0.3	0.85 ± 0.3
E/Em	9.7 ± 3.7	8.8 ± 2.9	10.2 ± 4.0	8.1 ± 2.0[c]	9.7 ± 3.9	8.7 ± 2.7	9.1 ± 3.3	9.5 ± 4.1
SBP (mmHg)	129 ± 16	126 ± 16	127 ± 19	125 ± 17	130 ± 15	125 ± 14[a]	129 ± 14	127 ± 16
DBP (mmHg)	81 ± 10	77 ± 10	79 ± 11	77 ± 10	81 ± 9	76 ± 9[a]	82 ± 11	78 ± 11
PWV (m/s)	10.2 ± 2.5	10 ± 2.2	10.05 ± 2.5	10.1 ± 2.3	11 ± 3	10.3 ± 2[a]	9.8 ± 1.9	9.6 ± 2.1
FMD (%)	5.4 ± 2.6	8.8 ± 3.1[b]	5.3 ± 3.0	10.5 ± 4.1[c]	5.9 ± 2.9	11.6 ± 3.6[c]	5.0 ± 1.9	4.3 ± 1.6[c]

ANOVA for paired comparisons using post hoc analysis with Bonferroni correction

GLS global longitudinal strain, *GLSR S* global longitudinal systolic strain rate, *GLSR E* global longitudinal early diastolic strain rate, *GLSR A* global longitudinal late diastolic strain rate, *CFR* coronary flow reserve, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PWV* pulse wave velocity, *FMD* flow-mediated dilation

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$ vs. baseline

Table 3 Markers of oxidative stress and inflammation

	All patients ($n=120$)		Anakinra ($n=40$)		Tocilizumab ($n=40$)		Prednisolone ($n=40$)	
	Baseline	3 months	Baseline	3 months	Baseline	3 months	Baseline	3 months
MDA, µM/L	2.0 (1.5–3.0)	1.5[a] (1.1–2.2)	2.2 (1.5–3.3)	1.5[a] (0.9–2.0)	1.8 (1.2–2.5)	1.0[b] (0.6–1.4)	2.0 (1.8–3.2)	1.9 (1.9–3.2)
PCs, nmol/mg	0.0132 (0.009–0.014)	0.0115[a] (0.007–0.013)	0.0131 (0.008–0.014)	0.0111[a] (0.007–0.012)	0.0136 (0.011–0.014)	0.0108[b] (0.008–0.014)	0.0129 (0.008–0.014)	0.0126 (0.007–0.013)
CRP, mg/L	12.4 (6.6–37)	3.5[b] (0.7–6.8)	12.6 (6.5–36)	3.1[c] (0.2–6.1)	13.7 (7.2–40)	2.3[c] (0.1–5.5)	10.9 (6.2–34)	5.2[a] (1.9-9)

ANOVA for paired comparisons using post hoc analysis with Bonferroni correction

MDA malondialdehyde, *PCs* protein carbonyls, *CRP* C-reactive protein

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$ vs. baseline

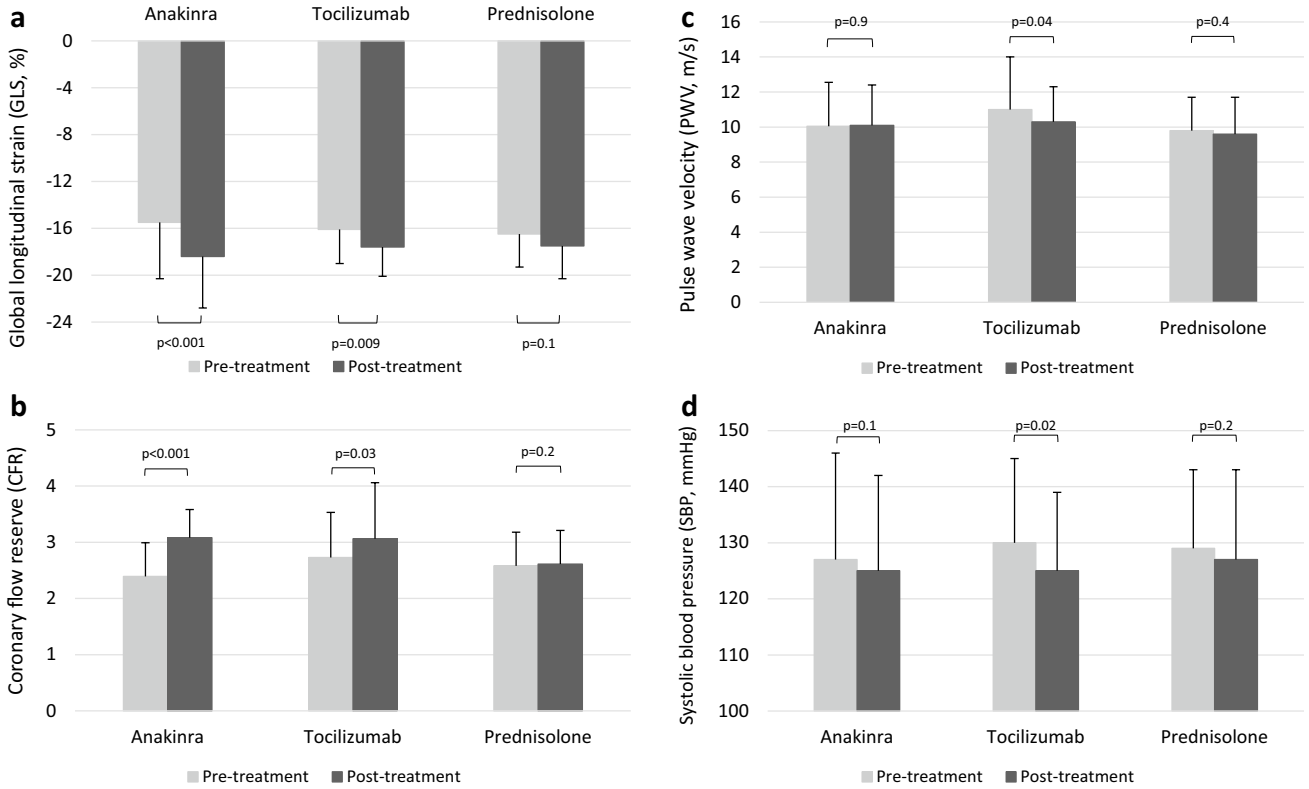

Fig. 1 **a** Global longitudinal strain (GLS, %), **b** coronary flow reserve (CFR), **c** pulse wave velocity (PWV, m/s), **d** systolic blood pressure (SBP, mmHg), in the three treatment groups pre- and post-treatment. The T lines on bars of the figures indicate standard deviation of the mean value

deterioration of flow-mediated dilatation was observed post-prednisolone (− 14%, p < 0.001).

Compared with anakinra and prednisolone, tocilizumab treatment resulted greater improvement of pulse wave velocity (− 7%, vs. 0.5% vs. − 2%, $F=5.1$, $p=0.04$, Table 2; Fig. 1c) as well as of both systolic and diastolic brachial blood pressure (− 4% vs. − 1.6% vs. − 1.6%, $F=6.1$, $p=0.03$ and − 6.5% vs. − 2.6% vs. − 4.6%, $F=5.2$, $p=0.04$, respectively, Fig. 1d).

All patients had reduced malondialdehyde, protein carbonyls, and C-reactive protein post-treatment ($p<0.05$, Table 3). However, there was a significant interaction between the type of treatment and changes in oxidative stress markers post-treatment ($p=0.03$, Table 3).

Compared with prednisolone, anakinra and tocilizumab resulted in a greater reduction of malondialdehyde (34% vs 39% vs 11%; $F=8.1$, $p=0.01$) and protein carbonyls (24% vs 26% vs 2%; $F=7.1$, $p=0.02$). The percent reduction

of malondialdehyde and protein carbonyls was similar between anakinra and tocilizumab ($p = 0.46$ and $p = 0.71$, respectively).

Compared with prednisolone, anakinra and tocilizumab resulted in a greater reduction of C-reactive protein (76% vs. 83% vs. 52%; $F = 9.1$, $p = 0.001$). The percent reduction of C-reactive protein was similar between anakinra and tocilizumab.

Total cholesterol, LDL cholesterol and triglycerides increased significantly in the tocilizumab group post-treatment compared to anakinra and prednisolone groups ($p < 0.05$, see Supplementary table).

Discussion

In the present study, we have shown that both inhibition of IL-1 activity by anakinra or IL-6 activity by tocilizumab-improved LV myocardial deformation markers, endothelial function, as assessed by coronary flow reserve and flow-mediated dilatation of the brachial artery, oxidative stress as assessed by malondialdehyde and protein carbonyls and inflammatory burden as assessed by C-reactive protein and disease activity score compared to corticosteroid treatment in patients with rheumatoid arthritis. However, in our study, LV myocardial deformation improved to a greater extent after inhibition of IL-1 activity by anakinra than after inhibition of IL-6 activity by tocilizumab. The effective inhibition of the direct detrimental effect of IL-1 on cardiomyocyte mitochondria [31] may explain the larger change of LV myocardial deformation post-anakinra compared to tocilizumab. Furthermore, the greater improvement of coronary flow reserve post-anakinra compared to tocilizumab may have also contributed to the greater improvement of global LV longitudinal strain after anakinra than after tocilizumab treatment. Moreover, the elevation of blood lipid levels observed after tocilizumab but not after anakinra treatment may have attenuated part of the beneficial effects of IL-6 inhibition on myocardial deformation and CFR. On the other hand, IL-6 inhibition resulted in a greater improvement of arterial stiffness compared to anakinra likely because of inhibition of the known detrimental effects of IL-6 activity on vascular smooth muscle cells and collagen turnover within the arterial layers [32–35].

Studies have shown an impaired coronary microcirculatory function as well endothelial dysfunction in rheumatoid arthritis [3, 36]. Adenosine-induced coronary flow reserve is at least partly endothelial dependent [3]. Myocardial dysfunction in rheumatoid arthritis is attributed to inflammation, enhanced nitro-oxidative stress [3, 37] and IL-1-mediated endothelin production [38]. Indeed, in the present study, both IL-1 and IL-6 inhibition improved coronary flow reserve likely because of a reduction of oxidative stress, inflammation as assessed by C-reactive protein and endothelial function as assessed by flow-mediated reserve. The greater effect of IL-1 compared to IL-6 inhibition on coronary flow reserve may be attributed to (a) the previously reported reduction of endothelin levels after IL-1 inhibition [3] and/or (b) the increase in blood lipids after tocilizumab, as found in the present study. The increase in lipid levels after tocilizumab is in line with previous studies showing that IL-6 inhibition increase total and LDL cholesterol blood levels by reducing various receptor surface levels and sPLA2-IIA levels leading to both decreased LDL and VLDL tissue retention and elevated circulating levels [8, 39–43].

IL-1 enhances the release of inflammatory cytokines with negative inotropic action including IL-6 [3, 44, 45] and promotes the release of superoxide anion [3], contributing to an enhanced nitro-oxidative stress resulting in a direct detrimental effect on myocardial mitochondria [31, 45]. Through the above mechanisms, IL-1 and IL-6 contribute to myocardial dysfunction. Indeed in the present as well as in our previous studies [3], we have shown the parallel improvement of oxidative stress and myocardial deformation after anti-inflammatory treatment.

However, the improvement of myocardial deformation was greater after anakinra than after tocilizumab despite a similar reduction of oxidative stress markers possibly because of (a) the direct beneficial effect of IL-1 inhibition on myocardium and intracellular oxidative stress and (b) the adverse increase of LDL cholesterol and triglycerides after tocilizumab treatment.

A prospective pilot study observed an improvement in endothelial function by flow-mediated dilatation and aortic stiffness after 3 and 6 monthly therapeutic infusions of tocilizumab for active rheumatoid arthritis [16]. In line with above findings, our study also demonstrated a similar improvement of flow-mediated dilatation after treatment with anakinra or tocilizumab. On the other hand, treatment with prednisolone resulted in a decrease of FMD in our study. Corticosteroids might induce negative effects on endothelial function via a direct effect on endothelium or via increasing glucose plasma levels and insulin resistance that reduce nitric oxide (NO) and impair arterial distensibility [46]. While most studies showed no deterioration of endothelial function after treatment with low dose or a bolus of corticosteroids [46], in our previous study, patients treated with higher doses prednisolone (> 5 mg, daily) had a borderline lower FMD after one month of treatment [3] as further confirmed in the current study during 3 months of treatment using high prednisolone doses (10 mg daily). Indeed, treatment with prednisolone doses > 8 mg daily have been associated with increased cardiovascular mortality in RA [47].

Furthermore, in our study, pulse wave velocity and brachial blood pressure were reduced only after tocilizumab and not after anakinra treatment despite a similar reduction of

oxidative stress markers, C-reactive protein and increase of flow-mediated dilatation in the two treatment arms. Previous studies have demonstrated that IL-6 stimulates production of matrix metalloproteinase and tissue inhibitor of metalloproteinase, and increases TGF-β receptor compartmentalization and turnover enhances TGF-β1 signaling [33, 34]. Thus, IL-6 results in accelerated vascular remodeling and fibrosis and increased arterial stiffness independently of IL-1 activity. Experimental studies have shown that inhibition of IL-6 activity prevents degradation of vascular smooth muscle cell contractile proteins α-SMA and SM22α and autophagy and thus restores aortic wall properties [35]. Thus, the direct effects of IL-6 inhibition by tocilizumab on vascular smooth muscle cells and collagen turnover may cause (a) a greater improvement of aortic wall properties as shown by the reduction of pulse wave velocity in this study leading to late arrival of wave reflection in diastole instead of systole and thus reducing central systolic aortic pressure [21, 29] and (b) an improved function of the medium and small size arterial vessel leading to reduced magnitude of wave reflections. The lower intensity wave reflections when they sum up with the central aortic pulse wave they generate a lower amplitude forward pulse wave to the peripheral arteries which in turn results in lower brachial blood pressure values after tocilizumab than after anakinra and prednisolone treatment.

Study limitations

The study design does not permit to explore the causality between the changes of the biomarkers with vascular and LV function post-treatment. From the current data, it is not clear whether baseline LV dysfunction is due to coronary microvascular impairment, increased arterial stiffness, negative inotropic effect of inflammatory markers or oxidative stress that track with disease activity. Finally, the fact that the study was monocentric, not blinded to patients and that there was no proof for actual compliance in regards of medical adherence except for the provision of empty ampoules should be acknowledged.

Conclusions

In patients with rheumatoid arthritis, both IL-1 and IL-6 inhibition by anakinra and tocilizumab, respectively, caused significant improvement on vascular and myocardial function compared to corticosteroid treatment likely through a similar profound reduction of oxidative stress and inflammatory burden. However, IL-1 inhibition by anakinra caused a greater improvement of LV myocardial strain and coronary flow reserve than IL-6 inhibition by tocilizumab. Conversely, IL-6 inhibition resulted in a greater improvement of arterial stiffness and blood pressure than IL-1 inhibition. Thus, the inhibition of interleukin 1 and 6 appears to have favorable effects on cardiovascular function. Indeed, recent studies suggest that inhibition of IL-1 [2, 48] and IL-6 may have a beneficial effect on cardiovascular outcome [49] in contrast to the use of nonselective nonsteroidal anti-inflammatory drugs [50].

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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