Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis

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Abstract

Background

Circulating interleukin (IL)-6 concentrations are associated with endothelial activation in rheumatoid arthritis (RA).

Objective

To assess endothelial activation before and after suppression of cytokine production in RA.

Methods

Twenty-one patients (mean (SD) age 59 (9) years; disease duration 6 (4) years) were treated with intraarticular methylprednisolone acetate (417 (152) mg) together with disease modifying agent (DMARD) initiation (n = 10) or intensification (n = 11) employing methotrexate (n = 11), leflunomide (n = 8), minocyclin (n = 6) and sulphasalazine (n = 1). Disease activity, circulating cytokines (IL-1, tumor necrosis factor alpha (TNF-α) and IL-6) and biomarkers of endothelial activation (circulating vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1)) were evaluated before and 2 weeks after treatment.

Results

The intervention resulted in reductions in 8 disease activity markers ($p \leq 0.002$). Serum IL-6 concentrations decreased from 17 (2.9) to 4.9 (4.6) pg/ml ($p = 0.0008$). Serum IL-1 and TNF-α levels did not change ($p \geq 0.4$). Serum VCAM-1 concentrations decreased from 912 (402) to 752 (252) ($p = 0.003$), ICAM-1 from 398 (205) to 323 (179) ($p = 0.04$) and ELAM-1 from 68 (28) to 53 (25) ($p = 0.02$) pg/ml, respectively. Baseline rheumatoid factor titers were associated with reductions in VCAM-1 ($r_s = 0.481$, $p = 0.03$). In multivariable regression models, decreases in circulating interleukin-6 concentrations were associated with reductions in VCAM-1 ($p < 0.0001$), ICAM-1 ($p = 0.005$) and ELAM-1 ($p = 0.02$) independent of changes in disease activity, weight and blood pressure.

Conclusion

Our results suggest that suppression of circulating IL-6 concentrations attenuates atherogenesis in active RA.

Key words

Rheumatoid arthritis, cytokine suppression, endothelial activation.

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Introduction

During the past six years, rheumatoid arthritis (RA) was found to be a risk factor for myocardial infarction in seven controlled studies (1-7). Apart from age and hypertension, other traditional risk factors do not seem to play a major role in cardiovascular (CV) disease in RA (2, 8). By contrast, the inflammatory cascade and its effects on traditional and novel CV risk factors are strongly implicated in RA atherogenesis (9,10). In addition, methotrexate use was reported to protect against CV death (11) and continuous oral glucocorticoid exposure was found to increase the risk for myocardial infarction (12) in RA.

Endothelial dysfunction is an essential initial step in atherogenesis (13). A variety of procedures has been recommended for the evaluation of endothelial function (14, 15). Investigators reported the presence of endothelial dysfunction as assessed by brachial artery flow-mediated dilatation (FMD) in RA (16-20). This was associated with HLA-DRB1*04 alleles in particular HLA-DRB1*0404 (16), circulating C-reactive protein (17), LDL cholesterol (17) and $CD4^{+}CD28^{null}$ T lymphocyte concentrations (18). While FMD depends on acute nitric oxide release (21, 22), circulating adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule (ELAM-1)) are biomarkers that reflect a different aspect of endothelial dysfunction, namely endothelial inflammation and activation (21, 23, 24). Of importance in the present context, circulating adhesion molecules do not originate in the synovium in RA (25). We have recently reported marked endothelial activation that is independently associated with circulating interleukin (IL)-6 concentrations in RA (24).

In the present study, we measured biomarkers of endothelial activation (21, 23, 24, 26-29) before and 2 weeks after treatment with intraarticular methylprednisolone acetate in combination with disease modifying agents (DMARD) in 21 patients with active RA. Intraarticularly administered methylprednisolone has potent and rapid disease activity and cytokine suppressant effects in RA (30, 31). In view of our previous findings (24), we hypothesized that suppression of cytokine production is associated with decreased endothelial activation in active RA.

Materials and methods

Patients

Twenty-one Caucasian patients who met the American Rheumatism Association criteria for RA (32) were enrolled. Their mean (SD) age and disease duration were 59 (9) and 6 (4) years, respectively; twenty (95%) were women. Exclusion criteria were the use of lipid lowering or antidiabetic medications and glucocorticoids over the previous 3 months. Eleven (52%) patients were taking stable doses of disease modifying agents for at least 3 months. These agents comprised methotrexate in 8, chloroquine in 2, minocyclin in 2, leflunomide in 1, azathioprine in 1 and sulphasalazine in 1 patient, respectively. Two patients had diabetes (fasting plasma glucose ≥ 7 mmol/l) (33) that was controlled on dietary intervention only. Twelve (75%) patients were hypertensive (blood pressure >140/90 mmHg or on antihypertensives). The study was approved by the local ethic committee for research on human subjects.

Baseline variable recording

Baseline characteristics were recorded using previously reported methods (8, 24) and are shown in Table I. Blood pressure measurements were made in accordance with reported guidelines on the evaluation and treatment of hypertension (34, 35). Fasting blood samples were taken between 0800 and 1000 hours am. Specimens were analyzed within 2 hours of blood sampling for the variables as recorded in Table I.

Enzyme-linked immunosorbent assays

IL-1, tumor necrosis factor-alpha (TNF-α), IL-6, VCAM-1, ICAM-1 and ELAM-1 were measured by enzyme-linked immunosorbent assays (Hiss Diagnostics, GmbH, Freiburg, Switzerland) on plasma specimens that had

been stored at -70° Celsius and using single assay runs. The intra-assay coefficients of variation were 5.1%, 6.9%, 3.4%, 3.1%, 4.1% and 5.4% for IL-1, TNF-α, IL-6, VCAM-1, ICAM-1 and ELAM-1, respectively.

Intervention

After the initial assessment, intraarticular methylprednisolone acetate (Depo-Medrol™, Pharmacia (Pty) Ltd., Midrand 1685, South Africa) was administered together with the long acting local anaesthetic agent bupivacaine (Macaine®, Adcock Ingram Ltd., Bryanston Extension 77, 2021, Johannesburg, South Africa) to all or most swollen joints using a mean (SD) methylprednisolone acetate total dose of 417 (152) mg. The number of joints injected per patient was 2 in 1, 3 in 2, 4 in 4, 5 in 1, 6 in 3, 7 in 2, 8 in 5, 9 in 1 and 10 in 2 patients, respectively. Five mg (1 ml) bupivacaine was added to each 40 mg (1ml) methylprednisolone acetate. Patients were advised to avoid activities for 24 hours following injections. The use of multiple intraarticular corticosteroid injections in active RA was reported previously (30, 36-40). On the same day, disease modifying agent therapy was initiated in 10 and intensified in 11 patients, respectively, employing methotrexate (n = 11), leflunomide (n = 8), minocyclin (n = 6) and sulphasalazine (n = 1). Concomitant medications were left unaltered and no dietary intervention was initiated during the study.

Follow-up evaluation

Two weeks subsequent to intraarticular methylprednisolone administration, disease activity parameters, body weight, blood pressure, plasma glucose and serum insulin concentrations and circulating cytokine and adhesion molecule levels were re-evaluated.

Statistical analysis

Continuous variables are presented as mean (SD) and categorical variables as number (%). For variables with skewed distributions geometric means are given. The Wilcoxon Signed Rank test was used to assess changes in recorded variables before and after treatment. Associations between changes in biomarkers and other baseline recorded variables were assessed by the Spearman correlation coefficient. Associations between changes in biomarkers and changes in other recorded variables were assessed in univariate and multivariable regression models. Variables with a skewed distribution were logarithmically transformed prior to entering them in regression models. P values of < 0.05 were considered significant.

Results

Baseline demographic, clinical and laboratory characteristics are summarized in Table I.

Effects of pulsed methylprednisolone and DMARD on disease activity, body weight, blood pressure, glucose metabolism, circulating cytokines and biomarkers of endothelial activation

Two weeks subsequent to enrollment, there was a marked reduction in each disease activity parameter (Table I). Also, mean body weight decreased by 3 kg and there were small reductions in systolic and diastolic blood pressure. Glucose and insulin levels did not change significantly. The 2 diabetic patients experienced a transient increase in plasma glucose levels that resolved within 2 days subsequent to methylprednisolone administration and

Table I. Baseline characteristics and results in 21 rheumatoid arthritis patients.

	Baseline	After treatment	*P*
Age (years)	59 (9)		
Women (n (%))	20 (95)		
Disease duration (years)	6 (4)		
DMARD users (n (%))	11 (52)		
NSAID users (n (%))	14 (67)		
Smoking (n (%))	8 (38)		
Exercising (n (%))	4 (19)		
Diabetes (n (%))	2 (10)		
Rheumatoid factor positive (n (%))	16 (76)		
Rheumatoid factor titer (IU/ml)	72 (9)		
Radiographic score	12 (5)		
LDL cholesterol (mmol/l)	2.9 (0.7)		
HDL cholesterol (mmol/l)	1.4 (0.4)		
Triglycerides (mmol/l)	1.4 (1.4)		
Uric acid (mmol/l)	0.30 (0.10)		
BMI (kg/m^2)	26.0 (5.9)		
Weight (kg)	67.6 (1.2)	64.6 (1.2)	0.0007
Glucose (mmol/l)	4.6 (1.2)	4.4 (1.2)	0.3
Insulin (uU/ml)	5.6 (2.1)	7.4 (1.6)	0.2
Systolic blood pressure (mmHg)	133 (15)	125 (17)	0.04
Diastolic blood pressure (mmHg)	85 (10)	79 (8)	0.01
HAQ-DI	1.6 (0.8)	0.7 (0.5)	0.002
VAS pain	6.5 (1.7)	1.5 (1.1)	< 0.0001
VAS patient disease activity	7.1 (1.5)	1.3 (1.3)	< 0.0001
VAS doctor disease activity	6.9 (1.5)	0.7 (0.7)	< 0.0001
Tender joints	17.3 (8.9)	0.7 (1.3)	< 0.0001
Swollen joints	8.7 (2.2)	0.2 (0.6)	< 0.0001
ESR (mm/hr)	43 (2)	12 (3)	< 0.0001
Hs-CRP (mg/l)	34.7 (2.8)	5.6 (2.6)	< 0.0001

Results are expressed as mean (SD) unless indicated otherwise.

Abbreviations: DMARD, disease modifying agents for rheumatic disease; NSAID, non-steroidal anti-inflammatory agents; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; HAQ-DI, Health Assessment Questionnaire-Disability Index (range 0-3); VAS, visual analog scale (range 0-10 for both pain, patient and doctor disease activity); ESR, erythrocyte sedimentation rate; hs-CRP, high sensitivity C-reactive protein.

did not require antidiabetic medications.

IL-6 and each of the 3 biomarkers of endothelial activation decreased 2 weeks subsequent to the intervention whereas IL-1 and TNF-α levels did not change significantly (Table II). The reductions in mean IL-6, VCAM-1, ICAM-1 and ELAM-1 concentrations were 71%, 18%, 19% and 22%, respectively. After treatment, the ELAM-1 concentrations were no longer higher than that previously reported by us in 80 healthy controls, i.e. 53 pg/ml (24).

Associations between baseline variables and reductions in biomarkers of endothelial activation

Baseline VCAM-1, ICAM-1 and ELAM-1 concentrations each predicted reductions in the respective biomarkers (r_s = 0.722, p = 0.0002 for VCAM-1, rs = 0.617, p = 0.003 for ICAM-1 and r_s = 0.540, p = 0.01 for ELAM-1). Further, baseline ICAM-1 levels predicted decreases in VCAM-1 (r_s = 0.514, p = 0.02) and the reductions in VCAM-1 and ICAM-1 were interrelated (r_s = 0.665, p = 0.01).

Baseline rheumatoid factor titers were associated with reductions in VCAM-1 (r_s = 0.481, p = 0.03). Baseline IL-6 concentrations were related to decreases in IL-6 (r_s = 0.682, p = 0.0007).

Baseline IL-6 and C-reactive protein concentrations, weight and blood pressure were not associated with decreases in VCAM-1, ICAM-1 and ELAM-1 (data not shown).

Associations between changes in recorded variables and reductions in biomarkers of endothelial dysfunction

Reductions in IL-6 were strongly associated with decreases in VCAM-1, ICAM-1 and ELAM-1, both before and after controlling for potential confounders comprising changes in CRP, weight and blood pressure (Table III). In the univariate analyses in Table III, reductions in IL-6 concentrations explained 88%, 36% and 28% of the variance (R^2 x 100) in decreases in VCAM-1, ICAM-1 and ELAM-1 levels, respectively. Changes in systolic blood pressure and diastolic blood pressure were collinear (r_s = 0.550, p = 0.01) and hence not entered simultaneously in multivariable regression models. Replacement of diastolic blood pressure by systolic blood pressure and hs-CRP by other disease activity markers did not materially alter the models in Table III. There were no significant associations between reductions in IL-6 and improvement in disease activity variables (data not shown).

Table II. Biomarkers of endothelial activation and cytokines before and after disease activity suppression in 21 rheumatoid arthritis patients.

	Before treatment	After treatment	*P*
VCAM-1 (pg/ml)	912 (402)	752 (252)	0.003
ICAM-1 (pg/ml)	398 (205)	323 (179)	0.04
ELAM-1 (pg/ml)	68 (28)	53 (25)	0.02
IL-1 (pg/ml)	3.4 (5.0)	5.0 (4.9)	0.4
TNF-α (pg/ml)	1.7 (5.4)	2.2 (5.9)	0.7
IL-6 (pg/ml)	17 (2.9)	4.9 (4.6)	0.0008

Results are expressed as mean (SD).
Abbreviations: IL, interleukin; VCAM-1, vascular adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; ELAM-1, endothelial leukocyte adhesion molecule-1.

Discussion

Reductions in IL-6 concentrations and decreased endothelial activation

In the present study, treatment with pulsed methylprednisolone in combination with DMARD resulted in a significant 18 to 22% reduction in the 3 measured biomarkers of endothelial activation in patients with active disease. Patients with marked endothelial activation at enrollment were most likely to experience an improvement in endothelial function. Reductions in IL-6 were strongly and independently associated with decreases in each of the 3 biomarkers of endothelial activation. By contrast, changes in IL-1, TNF-α and acute phase reactant concentrations were not associated with changes in any of the 3 biomarkers. Whereas IL-6

Table III. Univariate and multivariable regression models for reductions in VCAM-1, ICAM-1 and ELAM-1 before and after treatment.

	Δ VCAM-1*				Δ ICAM-1				Δ ELAM-1			
	Univariate		Multivariable		Univariate		Multivariable		Univariate		Multivariable	
	R^2	*P*	Partial R^2	*P*	R^2	*P*	Partial R^2	*P*	R^2	*P*	Partial R^2	*P*
Δ IL-6*	0.875	< 0.0001	0.871	< 0.0001	0.362	0.004	0.404	0.005	0.284	0.01	0.320	0.02
Δ hs-CRP*	0.011	0.65	0.002	0.85	0.001	0.85	0.021	0.57	0.015	0.59	0.009	0.96
Δ weight*	0.033	0.57	0.004	0.80	0.018	0.36	0.107	0.19	0.018	0.56	0.081	0.28
Δ diastolic BP	0.079	0.22	0.099	0.20	0.099	0.17	0.055	0.35	0.084	0.72	0.002	0.96
R^2 for model			0.890				0.477				0.343	

*Variables that were logarithmically transformed.
Abbreviations: VCAM-1, vascular adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; ELAM-1, endothelial leukocyte adhesion molecule-1; Δ, change or reduction in; R, correlation coefficient; hs-CRP, high sensitivity C-reactive protein; BP, blood pressure.

is a major *circulating* joint derived cytokine in RA, IL-1 and TNF-α are major proinflammatory cytokines in RA joints and induce IL-6 production (41-44). TNF-α or IL-1 blockade would therefore also be expected to decrease endothelial activation in RA. Indeed, the use of infliximab is associated with improved endothelial function as assessed by FMD (19, 20). Also, as was reported with methotrexate (11), TNF-α blockade was recently found to prevent CV events in RA (45). We have previously reported that circulating IL-6 concentrations are independently associated with endothelial activation in RA (24). In accordance with our current investigation, circulating IL-1, TNF-α and acute phase reactant concentrations were not associated with endothelial activation (24). Taken together, our previous findings (24) and current results are in keeping with the paradigm of cytokine mediated atherogenesis in RA (9, 10).

Baseline rheumatoid factor titers and decreased endothelial activation

In our previous report we found that, apart from circulating IL-6 levels, rheumatoid factor titers were also independently associated with endothelial activation (24). A potential role for humoral immunity in RA atherogenesis had also been suggested by previous investigators (46, 47). Rheumatoid factors are immunoglobulins that are produced by B cells (48). Low doses of glucocorticoids do not affect serum immunoglobulin levels or antibody synthesis in vivo after inoculation with various antigens (49). Indeed, in contrast to T cells, B cells are relatively resistant to the immunosuppressive effects of glucocorticoids (49). However, doses of methylprednisolone similar to those used in the present study and administered orally over 3 to 5 days were reported to profoundly reduce serum immunoglobulin levels in healthy subjects with a maximum suppression observed 2 to 4 weeks after treatment (50). This suppression resulted from an initial increase in immunoglobulin catabolism followed by decreased synthesis (50). Our current finding of an association between high baseline rheumatoid factor concentrations and reductions in endothelial activation further supports the role of humoral immunity in RA atherogenesis.

Changes in blood pressure and body weight with cytokine suppressant therapy

The decreases in blood pressure and body weight with pulsed methylprednisolone in combination with DMARD were unexpected. In view of the rapid disappearance of methylprednisolone from the circulation (31, 51), potential mineralocorticoid effects should have worn off 2 weeks subsequent to its administration. While intraarticular methylprednisolone has strong inhibitory effects on cytokine production (31), proinflammatory cytokines upregulate various components of the renin-angiotensin system (52) and renin-angiotensin activation has been documented in RA (53, 54). Fluid retention during arthritic episodes in palindromic rheumatism has also been reported (55). In addition, decreased energy expenditure upon physical activity that may be cytokine related was recently described in RA (56). Finally, the marked decrease in disability in our patients may have allowed them to be more physically active. Changes in blood pressure and body weight were not predictive of reductions in circulating biomarkers of endothelial dysfunction.

Intraarticular corticosteroids in RA

We used relatively high doses of methylprednisolone acetate in view of the marked baseline disease activity in our patients (for example, the mean Health Assessment Questionnaire-Disability Index was 1.6 and the mean swollen joint count was 9). However, these doses are still substantially lower than those used in reported investigations on intravenously administered pulsed methylprednisolone (typically 1 to 3 grams) in patients with active RA (eg., 57-59) whereas such high doses were reported to contribute to insulin resistance in RA (33). We therefore elected the intraarticular route in the present investigation. Our study was also not designed to determine the differential effects of pulsed methylprednisolone and DMARD on endothelial activation. Although classical DMARD typically take one or more months to suppress the inflammatory cascade in RA joints (60), whether these agents could have direct and more rapid effects on the vascular system is unknown at this stage.

We have previously reported a marked reduction in insulin levels with the use of pulsed glucocorticoids and traditional DMARD (30). In that study, patients were assessed at baseline and after 2 months (30). In the present investigation, insulin concentrations did not change significantly. The 2 diabetic patients demonstrated a transient increase in their plasma glucose concentrations. We suggest that pulsed glucocorticoids exacerbate insulin resistance in RA but that this effect is transient and reversible.

Study limitations

Our study has potential limitations. Endothelial function was not assessed by FMD. Biomarkers of endothelial dysfunction are equally promising tools in the assessment of CV risk in RA (24, 61, 62). We did not study a placebo treated control group. Withholding treatment in active RA is no longer considered ethical (19, 63, 64). Our findings are based on objective tests and the investigators who performed the laboratory tests were blinded to the study protocol. Potential long term adverse effects of methylprednisolone pulse therapy on endothelial function in RA need to be considered. The reported finding that intraarticularly administered methylprednisolone diffuses from the joints into the systemic circulation for only 7 days (31, 51) together with the improvement in endothelial function 2 weeks subsequent to its administration in the present investigation make it highly unlikely that this intervention has long term adverse effects on endothelial function in RA. Future studies should include evaluation of all potential CV risk factors such as lipids and rheumatoid factor titers, not only before but also after treatment. The effects of the current in-

tervention on lipids and insulin sensitivity were the subject of a previous report (30). We used a different DMARD regimen in each patient and this may have affected our results. Finally, the long term effects of cytokine suppression on endothelial activation in RA need further investigation.

Conclusion

In conclusion, the use of pulsed methylprednisolone and DMARD in active RA resulted in reduced endothelial activation that was most pronounced in patients with high rheumatoid factor concentrations at baseline, thereby supporting a potential role of humoral immunity in RA atherogenesis. Decreases in circulating IL-6 concentrations were consistently and independently associated with reductions in endothelial activation. Our findings suggest that suppression of IL-6 production attenuates atherogenesis in RA.

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