The role of endocan and selected pro-inflammatory cytokines in systemic lupus erythematosus

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Abstract

Introduction: Systemic lupus erythematosus (SLE) is a multisystem inflammatory autoimmune disease with a wide spectrum of clinical manifestations. Cytokines such as interleukin-1 (IL-1) and tumour necrosis factor α (TNF-α) are involved in its pathogenesis. Endocan is a novel marker of endothelial dysfunction and is likely to be engaged in proinflammatory processes in SLE.

Aim: To determine whether endocan serum concentration in SLE patients vary from healthy controls.

Material and methods: The study included 36 patients with SLE. SLEDAI-2K score was used to assess disease activity. The control group comprised 23 healthy volunteers. ELISA kits were used to assess serum concentrations of endocan, IL-1β, TNF-α, vascular endothelial growth factor (VEGF) and high-sensitivity C reactive protein (hs-CRP).

Results: The serum concentration of endocan was significantly higher ($p < 0.001$) in the SLE group than in healthy individuals. A positive correlation was found between serum levels of endocan and IL-1β ($r = 0.47$, $p < 0.05$). Active SLE patients (SLEDAI-2K score above 6 points) with an elevated total cholesterol level (above 5.17 mmol/l) were found to have VEGF concentration higher than those with a normal cholesterol level ($p < 0.03$). No other relevant relationships were found between the serum concentration of endocan, other laboratory parameters, anthropometric features, activity and duration of SLE.

Conclusions: A higher serum level of endocan in SLE patients indicates its possible role in the pathogenesis of the disease and reflects endothelial dysfunction. Our findings indicate that endocan could serve as a potential marker of endothelial dysfunction in SLE.

Key words: endocan, interleukin-1, tumour necrosis factor, vascular endothelial growth factor, systemic lupus erythematosus.
Aim

The aim of the study was to determine whether endocan serum concentration in SLE patients vary from healthy controls.

Material and methods

The study involved 36 patients (32 females and 4 males) aged between 29 and 80 years (mean: 49.6 years). The diagnosis of SLE was based on the revised criteria of the American College of Rheumatology (ACR) and ACR/EULAR criteria as they are still widely accepted [4, 5]. The mean duration of the disease was 14.7 years (range: 1–36 years). The study included patients with active and those with inactive disease. In all patients, the activity of the disease was determined according to SLEDAI-2K score [6]. Although maximum score in this system is 105 points, the number of points in the present group ranged from 0 to 15. In the present study, a score of 6 or above was considered as active disease, and a score below 6 as inactive disease. The control group comprised 24 healthy volunteers (16 females and 8 males) aged between 22 and 59 years (mean: 30.9 years). The hematological and biochemical parameters of the healthy individuals were within normal ranges.

Neither the patients with SLE nor the controls showed any clinical signs of infectious or neoplastic disease. They had not received antibiotics or any other antibacterial or antiviral medications for at least 4 weeks before blood collection.

Each participant underwent a thorough physical evaluation by one of the authors (T. K.). Blood samples from the patient and control groups were collected in pyrogen-free tubes containing anticoagulant at the time of the clinical assessment. The obtained serum was divided into aliquots and stored at –20°C until assayed for endocan, IL-1β, TNF-α, vascular endothelial growth factor (VEGF) and high-sensitivity C reactive protein (hs-CRP). The sera were randomly coded and testing was carried out without knowledge of the clinical status of the subject or of related laboratory data. Commercially available ELISA kits were used to assess the serum concentrations of endocan (ELAb Science, China), IL-1β, TNF-α, VEGF (R&D systems Inc. USA) and hs-CRP (Demeditec Diagnostics GmbH, Germany), in accordance with the manufacturer’s instructions. The analysis was performed using an E 960-ELISAreader at 450 nm (Metertech, USA).

The sensitivity of the assay was found to be 6.2 pg/ml for endocan, 1 pg/ml for IL-1β, 1.6 pg/ml for TNF-α, and 9 pg/ml for VEGF.

On the day of blood sampling for cytokines, the following laboratory parameters were analysed: complete blood cell count, urine analysis, urea and creatinine levels, glucose level, liver function tests (AST, ALT), antinuclear antibodies (ANA), total cholesterol level, LDL and triglyceride level. Informed consent was obtained prior to the study from all patients and healthy individuals participating in the study. The study was approved by the local ethics committee (RNN/71/17/KE).

Statistical analysis

All analyses were performed with Statistica, version 12 (StatSoft, Poland). As the Shapiro-Wilk test found the measured parameters not to have a normal distribution, nonparametric tests were used. The Mann-Whitney U-test was used to compare results between two groups, and Spearman’s rank correlation was used to measure the statistical dependence between two parameters. Results were displayed as median with lower (25th) and upper (75th) quartile (25th–75th centile). In all calculations, a p-value less than 0.05 was regarded as statistically significant.

Results

In the SLE group, 9 patients were in the active stage (from 6 to 15 points, mean: 10.77) of the disease and 27 in the inactive stage according to SLEDAI-2K (< 6 points, mean: 4.34). The mean body mass index (BMI) was 23.8 kg/m² in the SLE patients and 22.9 in the group of healthy volunteers. The clinical and laboratory features of SLE patients are presented in Table 1. The biochemical parameters in SLE patients are shown in Table 2.

The serum concentrations of endocan, IL-1β, TNF-α, VEGF, and hs-CRP in SLE patients are shown in Table 3.

The mean serum concentration of endocan in the SLE group was 128 pg/ml and it was significantly higher (p < 0.001) than in healthy individuals (64.3 pg/ml). In addition, a positive correlation was found between serum levels of endocan and IL-1β (r = 0.47, p < 0.05). The mean serum concentration of IL-1β was 3 pg/ml in the SLE group, but 1.8 pg/ml in the control group, that of TNF-α was 7 pg/ml in the SLE group and 5.3 pg/ml in the control group, and that of VEGF was 421 pg/ml in the SLE patients and 338 pg/ml in the control group. Higher VEGF concentrations were observed in SLE patients with an elevated total cholesterol level (above 5.17 mmol/l) than in those with normal levels (p < 0.03). Hs-CRP serum concentration was significantly higher in the SLE patients (6 μg/ml) than in the group of healthy volunteers (2.2 μg/ml) (p < 0.005) (Figures 1, 2).

Discussion

SLE is a chronic inflammatory autoimmune disease with multiple organ involvement, which demonstrates a chronic course and variable severity, with a waxing and waning sequence. In some patients, particularly those who do not receive treatment, the outcome can be fatal. Early tissue damage is mostly related to the disease itself, whereas the later damage is usually caused by infections, atherosclerosis and malignancies. Those factors are usually related to complications associated with longstanding disease and immunosuppressive therapy.
that focus on cardiovascular risk assessment tend to be
coronary artery disease. Traditional cardiovascular risk
disease in patients with SLE. Atherosclerotic plaque has
disease in SLE patients was approximately seven to eight
times greater than that observed in the general popula-
ction.

Results are displayed as median with the range and mean ± standard devia-

Investigators report that the incidence of cardiovascular
disease in SLE patients was approximately seven to eight
times greater than that observed in the general popula-

Table 1. Clinical and laboratory characteristics of SLE
patients according to updated American College of
Rheumatology Criteria for Classification of Systemic
Lupus Erythematosus

| Symptoms                        | Number of patients | %   |
|---------------------------------|--------------------|-----|
| Malar rash                      | 31                 | 86.1|
| Discoid rash                    | 7                  | 19.5|
| Photosensitivity                | 35                 | 97.2|
| Oral ulcers                     | 7                  | 19.5|
| Nonserosive arthritis           | 26                 | 72.2|
| Pleuritis or pericarditis       | 0                  | 0   |
| Renal disorders                 | 5                  | 13.8|
| Hematologic disorders           | 25                 | 69.4|
| Immunologic disorders           | 8                  | 22.2|
| Positive antinuclear antibody   | 36                 | 100 |

Table 2. Biochemical and laboratory characteristic of SLE
patients

| Parameter                        | Number of patients | %   |
|----------------------------------|--------------------|-----|
| Total cholesterol level above 5.17 mmol/l | 10                 | 27  |
| LDL cholesterol level above 2.6 mmol/l  | 6                  | 16.6|
| Triglycerides level above 1.69 mmol/l  | 4                  | 11  |
| Aspartate aminotransferase level above 40 U/l | 2                  | 5.5 |
| Alanine aminotransferase level above 56 U/l  | 3                  | 8.3 |
| Glucose level above 5.5 mmol/l      | 3                  | 8.3 |
| ds-DNA positive                    | 9                  | 25  |
| Active SLE                         | 9                  | 25  |
| Inactive SLE                       | 27                 | 75  |

Table 3. Serum levels of endocan, IL-1, TNF-α, VEGF, hs-CRP in patients with SLE and healthy control subjects.

| Molecule      | SLE patients, n = 36 | Healthy controls, n = 23 |
|---------------|-----------------------|--------------------------|
| Endocan       | 122.2 (96.15–164.70) pg/ml | 54 (29–85) pg/ml |
|               | 128 ±49.2 pg/ml        | 64.3 ±54.5 pg/ml         |
| IL-1β         | 1.5 (1.3–1.75) pg/ml   | 1.7 (1.3–2) pg/ml        |
|               | 3 ±6.6 pg/ml           | 1.8 ±0.8 pg/ml           |
| TNF-α         | 6.4 (5.5–7.6) pg/ml    | 5.3 (0.9–2) pg/ml        |
|               | 7 ±3.1 pg/ml           | 5.3 ±1.2 pg/ml           |
| VEGF          | 274.45 (168.5–546.86) pg/ml | 256.9 (160–456) pg/ml |
|               | 421 ±342.3 pg/ml       | 338 ±258.2 pg/ml         |
| hs-CRP        | 4.1 (1.4–10.5) µg/ml   | 0.5 (0.1–3) µg/ml        |
|               | 6 ±5.1 µg/ml           | 2.2 ±3.3 µg/ml           |

Results are displayed as median with the range and mean ± standard deviation.

data indicate that such endothelial dysfunction may result from chronic inflammation and that it may con-
tribute to the pathogenesis of many autoimmune dis-
eases of the connective tissue. It is worth mentioning
that only a single study has so far examined the role of
endocan in SLE pathogenesis: Icli et al. found the level
of serum endocan to be higher in the tested group of
SLE patients than in controls and proposed that endocan
might be an useful marker of subclinical atherosclerosis
in lupus patients. They also highlighted the existence of
a relationship between endocan concentration and car-
rotid intima-media thickness (cIMT): a marker of early-
stage atherosclerosis [10].

Endocan is expressed in various tissues, including
the skin. Its level was found to be elevated in the serum
of patients with psoriasis vulgaris, in whom endocan
concentration also correlated with cIMT [11]. Moreover,
endocan has been proposed as a potential biomarker for
other autoimmune diseases such as systemic sclerosis
[12], Behcet’s disease [13], inflammatory bowel diseases
[14] and rheumatoid arthritis [15]. Our findings confirm
that the level of endocan is significantly elevated in SLE
patients; however no correlation was found with disease
activity, which might be associated with the small differ-
ence in SLEDAI 2K score observed in the present study
between patients with active (10.77) and inactive courses
(4.34) of the disease.

It is also believed that endocan could serve as an in-
dicator of angiogenesis and endothelial cell activation.
However, its activity in SLE patients has not been thor-
oughly evaluated so far, and very little is known about
its relationship with other proinflammatory cytokines.
Despite this, its action is thought to be regulated by
multiple cytokines, such as TNF-α, IL-1β and VEGF [3, 11,
16, 17]. TNF-α and IL-1β stimulate its secretion, and IFN-γ
inhibits the stimulation induced by TNF-α. Moreover,
expression of endocan is significantly increased in the pres-
The importance and clinical significance of angiogenic cytokines in the pathogenesis of collagen diseases has been less intensively investigated than in neoplastic diseases. Vascular endothelial growth factor (VEGF) is a glycoprotein produced by endothelial cells, macrophages, and vascular smooth muscle cells. It plays a key role in the development of inflammatory response, induces local inflammation, and promotes angiogenesis and endothelial cell proliferation. VEGF also plays a role in the regulation of vascular permeability, recruitment of leukocytes, and the promotion of angiogenesis.

In the present study, a positive correlation was found between IL-1β and endocan in the SLE group, despite the fact that similar IL-1β levels were observed in the SLE patients and healthy volunteers. This relationship may indicate that inflammation and endothelial dysfunction might interact in the pathogenesis of SLE.

IL-1β and TNF-α are regarded as representative multifunctional proinflammatory cytokines, participating not only in the development of inflammatory response, but also in immune system dysregulation. Both cytokines are thought to play an important role in the immunopathology of SLE. There are many studies indicating that IL-1β plays a key role in the disease pathogenesis and can serve as a potential therapeutic target. The major function of IL-1β is to act as a mediator of the inflammatory reaction to infections. Depending on its serum level, IL-1β promotes local inflammation and induces the production of such mediators as prostaglandins, nitric oxide, and cyclooxygenase 2. At higher concentrations, IL-1β can induce fever and acute-phase proteins. Its role in the pathogenesis of SLE is widely documented in the literature. Together with TNF-α, IL-1β promotes selective expression of intercellular adhesion molecule 1 (ICAM-1) on the vascular endothelium, which plays an important role in the process of leukocyte adhesion. The interaction of these two cytokines stimulates the development of the inflammatory reaction observed in SLE.

Our study found the SLE patients to have a higher mean serum level of IL-1β than controls (3.0 vs. 1.8 pg/ml); however, no significant relationship was observed with disease activity, which may result from the small number of studied patients.

Tumour necrosis factor α (TNF-α) exerts many physiological and pathogenic effects in the human body. It contributes to the development of immunocompetent cells, promotes apoptosis, plays an important role in the inflammatory processes and is also involved in many biochemical and signalled pathways. TNF-α has been reported to play a major pro-inflammatory role in SLE; however, while the TNF-α level was found to be higher in SLE patients than in healthy volunteers in the present study, this difference was not significant.

An interesting observation is that one of the patients with the highest serum concentration of endocan (227.2 pg/ml) also presented the highest levels of TNF-α (17.9 pg/ml) and hs-CRP (15.2 μg/ml), suggesting that endocan could play a role in the inflammatory pathways associated with the course of lupus. The TNF-α serum level did not differ significantly between the active and inactive stage of the disease, which can also be related to the small differences in SLEDAI-2K score between the two groups. Although most studies indicate that the serum level of TNF-α correlates with disease activity and remains higher than in controls, others have found that elevated TNF-α plasma levels did not show any correlation with SLE activity or that the TNF-α serum level was higher in patients with inactive disease, with the authors suggesting that TNF-α may in fact play a protective role in lupus.

Inaccuracies may also result from the use of small groups of participants. The importance and clinical significance of angiogenic cytokines in the pathogenesis of collagen diseases has been less intensively investigated than in neoplastic diseases. Vascular endothelial growth factor (VEGF) is a glycoprotein produced by endothelial cells, macrophages,
fibroblasts, smooth muscle cells and cancer cells. It has the ability to bind to endothelial cell receptors (VEGFR-1 and 2), which leads to the induction of angiogenesis. Some studies have confirmed that the level of VEGF is elevated in SLE patients [2] and may be correlated with disease activity [25, 26]. Our present findings suggest that the mean VEGF concentration was higher in SLE patients than in healthy volunteers; however, these differences were not statistically significant. Also, no differences in the VEGF level were found between patients with active and inactive SLE. Again, this may be associated with the fact that only small differences could be observed between the groups according to SLEDAI-2K score.

An interesting positive relationship was found between VEGF and the presence of a total cholesterol level above 5.17 mmol/l; this could serve as evidence for the participation of VEGF in the development of atherosclerosis in patients with SLE. Krejca et al. report a positive correlation between the elevated VEGF level in serum and the total cholesterol level in patients with coronary artery disease [27]. A similar correlation has been found elsewhere in patients with diabetes mellitus; the VEGF serum level was associated with the elevated total cholesterol level and demonstrated a positive correlation with diabetic retinopathy and nephropathy [28]. Further investigations focused on VEGF and hypercholesterolemia may contribute to a better understanding of vascular dysfunction and the development of atherosclerosis in lupus.

C-reactive protein (CRP) is an acute phase reactant which is used as a marker of inflammation and tissue destruction [29]. However, while elevated CRP is generally a hallmark of inflammation, its level is not always elevated in lupus patients; in fact, many SLE patients demonstrate normal or even reduced levels of CRP. CRP is involved in the clearance of apoptotic cells, and their inadequate clearance can expose nuclear antigens to antinuclear antibodies with subsequent formation of immune complexes [30]. The usage of high sensitivity CRP can be used to assess even low CRP levels during chronic inflammation. Chronic inflammation has been recognized as an element in the progression of atherosclerosis [31].

CRP can serve as a useful marker for chronic inflammation in the course of SLE, which is an element in the pathology of connective tissue diseases, even where no clinical evidence is present; furthermore, studies have shown it to also be an accurate marker of an elevated risk of cardiovascular events [32]. In our study, the hs-CRP level was found to be significantly higher in SLE patients than in the group of healthy controls. These findings may contribute to a better understanding of the significance of chronic inflammation as a factor in the pathogenesis of SLE.

Conclusions

A higher serum level of endocan was observed in SLE patients than in controls, which indicates that it may play a role in the pathogenesis of the disease. The findings suggest that endocan could serve as a potential marker of endothelial dysfunction.

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Conflict of interest

The authors declare no conflict of interest.

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