
INTERLEUKIN-6 IS A POTENTIAL TARGET FOR A CORRECTION OF ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH LOW-GRADE SYSTEMIC INFLAMMATION

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Abstract

Introduction: One of pathogenetic links of development of endothelial dysfunction, as an early marker of cardiovascular disease and comorbidity, is a low-grade systemic inflammation. In this regard, correction of cytokines imbalance is one of the possible ways of prevention and treatment of major chronic noninfectious diseases.

Aims: To study the endothelium protective effects of anti-IL-6 receptor monoclonal antibodies tocilizumab in experimental preclinical studies in a model of endothelial dysfunction associated with the low-grade systemic inflammation.

Methods: The study was performed with 40 white male Wistar rats. The endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress was produced by sequential administration of gentamicin, bacterial lipopolysaccharide and N-nitro-L-arginine methyl ester. The administration of tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) was performed at 6 day from the experiment start in doses of 4 mg/kg and 8 mg/kg once. The endothelium protective activity was studied at 34th day from the start through the analysis of changes of pharmacological vascular tests and calculation of the endothelial dysfunction coefficient. The cytokine profile was assessed by measuring of serum concentrations of tumor necrosis factor-α, interleukin-6 and interleukin-10 by enzyme linked immunosorbent assay.

Results: In the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress, tocilizumab at the doses of 4 mg/kg and 8 mg/kg had the dose-dependent endothelium protective action that is logged to reduce of the endothelial dysfunction coefficient from 7.2±0.6 to 2.8±0.3 and 2.3±0.2, respectively. In addition, there was normalization of the cytokine profile in the form of lower serum concentrations of the tumor necrosis factor-α, interleukin-6 and interleukin-10.

Conclusion: Tocilizumab (at the doses of 4 mg/kg and 8 mg/kg) after one dose delivery has a pronounced endothelium protective activity in the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress and prevents of the cytokine profile imbalance.

Key words: endothelial dysfunction, low-grade systemic inflammation, interleukin-6, tocilizumab.

Introduction

Endothelial dysfunction is an early marker and predictor of cardiovascular diseases [1, 2, 3, 4, 5]. It is characterized by dysfunction of relaxation caused by nitric oxide (NO) and other vasodilatory compounds (such as prostacyclin), whereas the production of endothelial vasoconstrictor factors increases [6, 7, 8]. Pro-oxidant and proinflammatory vascular environment is another endothelial dysfunction characteristic [8, 9].
Low-grade chronic inflammation has a close pathogenetic connection with the development of endothelial dysfunction. Key molecules that trigger the main pathogenetic links are C-reactive protein and proinflammatory cytokines such as tumor necrosis factor alpha, interleukins 1β and 6 [1, 6, 10, 11]. The increase of their concentration in the serum may lead to reduction of NO production by endothelial nitric oxide synthase (eNOS) inhibiting [6, 12, 13], and the increasing of adhesion molecules expression, stimulating the migration of white blood cells and initiate the proliferation of smooth muscle cells of blood vessels [14, 15].

In this regard, the search and preclinical studies of drugs with endothelium protective properties aimed at correcting the cytokines imbalance are one of the most promising areas of targeted therapy.

Materials and methods

Animals

The study was performed with 40 white male Wistar rats weighing 220±10 g. Animals were kept under controlled environmental conditions when the air temperature is +18-25°C, humidity is 40-60%, under a 12-hour lighting cycle. Plan and research protocol were approved by the local ethics committee (protocol №12-2016 from 21.11.2016). Studies were conducted in compliance with ethical norms and rules with regard to the requirements of the European Convention for the protection of vertebrate animals using for experimental and other scientific purposes (1986), principles of good laboratory practice, state standard 33044-2014 and the order of the Ministry of health of the Russian Federation No. 199n «On approval of Rules of good laboratory practice».

Experimental groups

All animals were divided into 4 groups of 10 animals each (table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Intact animals</td>
</tr>
<tr>
<td>B</td>
<td>A control group with the modeling of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress</td>
</tr>
<tr>
<td>C</td>
<td>A group with tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) administration at the dose of 4 mg/kg on the background of endothelial dysfunction</td>
</tr>
<tr>
<td>D</td>
<td>A group with tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) administration at the dose of 8 mg/kg on the background of endothelial dysfunction</td>
</tr>
</tbody>
</table>

The endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress was modulated by the following way: gentamicin at a dose of 20 mg/kg was administrated intraperitoneally once a day, for 5 days. After administration of gentamicin, there was started the administration of the E. coli lipopolysaccharide type O55:B5 at a dose of 1 mg/kg intraperitoneally once every week for three weeks. At the 27th day from the experiment start the animals of the experimental group were intraperitoneally injected with N-nitro-L-arginine methyl ester (L-NAME) at a dose of 25 mg/kg daily for week.

The administration of tocilizumab was performed intraperitoneally once at 6 day after the experiment start in doses of 4 and 8 mg/kg, respectively.

Evaluation of hemodynamic and pharmacological vascular tests

At the 34th day from the experiment start under anesthesia (chloral hydrate 300 mg/kg) there was catheterized the left carotid artery for registration of hemodynamic parameters: systolic, mean and diastolic arterial pressure (SBP, MAP and DBP), measuring by the sensor TSD104A and hardware and software MP150 complex produced by «Biopac System inc.», the USA. There were conducted functional tests: endothelium-dependent vasodilatation (EDVD) after intravenous administration of acetylcholine (AC) at a dose of 40 mcg/kg and endothelium-independent vasodilatation (EIVD) after intravenous administration of nitroprusside sodium (NP) at a dose of 30 mg/kg.

To evaluate the severity of endothelial dysfunction there was used the endothelial dysfunction coefficient (EDC), calculated as the ratio of the areas of the triangles above the curve recovery of blood pressure after nitroprusside and acetylcholine administration.

The evaluation of cytokine profile

Blood samples from each rat was centrifuged at 3000 rpm for 15 minutes, the plasma was...

Statistical data processing
Statistical analysis of obtained data was performed in Microsoft Excel. "Descriptive statistics" was used to calculate the mean value (M) and standard error of the mean (m). "The two-sample t test with unequal variances" was used to compare the data of different groups of animals and determining the significance of differences between them. Statistically significant differences were considered when p<0.05.

Results
The results of the assessment of hemodynamic and vascular pharmacological tests
Modeling of the endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress, caused arterial hypertension (SBP – 173.0±15.2, DBP – 134.0±3.9, MAP – 152.0±11.2 mm Hg) and led to a greater loss of the arterial blood pressure after the acetylcholine administration (SBP to 102.9±8.1, DBP to 66.9±8.0, MAP to 79.2±4.8 mm Hg) and less loss of it after the nitroprusside administration (SBP to 92.8±4.7, DBP to 47.1±4.2 and MAP to 62.0±2.9 mm Hg) in comparison with the intact animals.

Tocilizumab administration dose-dependently reduced the severity of hypertension, however, did not allow to achieve the values of the intact animals group (table 2). On the other hand, processing of the experimental data allowed to establish that tocilizumab has the strong endothelium protective action, expressed in a statistically significant decrease of the endothelial dysfunction coefficient to 2.8±0.3 and 2.3±0.2 in the animal groups receiving the drug at the doses of 4 and 8 mg/kg, respectively (table 3).

Table 2
Dynamics of hemodynamic parameters in the experimental animal groups (M ± m; n = 10)

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (raw values)</td>
<td>137±3.7</td>
<td>101.9±4.3</td>
<td>115.0±1.9</td>
</tr>
<tr>
<td>EDVD</td>
<td>84.3±4.4</td>
<td>38.7±2.8</td>
<td>53.9±2.7</td>
</tr>
<tr>
<td>EIVD</td>
<td>83.0±3.7</td>
<td>42.1±4.4</td>
<td>55.7±3.5</td>
</tr>
<tr>
<td>B (raw values)</td>
<td>190.3±6.7*</td>
<td>145.0±3.9*</td>
<td>160.1±4.6*</td>
</tr>
<tr>
<td>EDVD</td>
<td>110.6±5.2*</td>
<td>82.8±6.6*</td>
<td>92.1±6.1*</td>
</tr>
<tr>
<td>EIVD</td>
<td>88.7±4.7*</td>
<td>50.8±4.2*</td>
<td>63.4±4.1*</td>
</tr>
<tr>
<td>C (raw values)</td>
<td>157.9±6.9**</td>
<td>133.0±4.1**</td>
<td>141.6±4.4</td>
</tr>
<tr>
<td>EDVD</td>
<td>86.1±3.8**</td>
<td>47.2±3.6**</td>
<td>59.0±3.2</td>
</tr>
<tr>
<td>EIVD</td>
<td>94.6±3.1**</td>
<td>43.9±0.9**</td>
<td>60.8±1.2</td>
</tr>
<tr>
<td>D (raw values)</td>
<td>153.9±4.5**</td>
<td>128.0±1.7**</td>
<td>138.2±2.0</td>
</tr>
<tr>
<td>EDVD</td>
<td>79.9±2.6**</td>
<td>56.4±2.1**</td>
<td>64.2±1.9</td>
</tr>
<tr>
<td>EIVD</td>
<td>105.5±3.7**</td>
<td>45.9±2.1**</td>
<td>65.8±1.8</td>
</tr>
</tbody>
</table>

Comment: SBP – systolic blood pressure; DBP – diastolic blood pressure; MAP – mean arterial pressure; EDVD – endothelium-dependent vasodilatation; EIVD – endothelium-independent vasodilatation; * – p<0.05 in comparison with the intact animals; ** – p<0.05 in comparison with the control group.

Table 3
The calculation of the endothelial dysfunction coefficient in the experimental animal groups (M ± m; n = 10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Functional tests</th>
<th>The area of the vascular response (relative units, RU)</th>
<th>EDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>EDVD</td>
<td>1268.0±74.8</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td></td>
<td>EIVD</td>
<td>1375.3±93.7</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>EDVD</td>
<td>814.2±78.0*</td>
<td>7.2±0.6*</td>
</tr>
<tr>
<td></td>
<td>EIVD</td>
<td>5862.3±116.7*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>EDVD</td>
<td>1119.4±110.1**</td>
<td>2.8±0.3**</td>
</tr>
<tr>
<td></td>
<td>EIVD</td>
<td>3006.5±232.5**</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>EDVD</td>
<td>1253.3±71.5**</td>
<td>2.3±0.2**</td>
</tr>
<tr>
<td></td>
<td>EIVD</td>
<td>2823.2±114.3**</td>
<td></td>
</tr>
</tbody>
</table>

Comment: EDVD – endothelium-dependent vasodilatation; EIVD – endothelium-independent vasodilatation; EDC – endothelial dysfunction coefficient; * – p<0.05 in comparison with the intact animals; ** – p<0.05 in comparison with the control group.
The results of the evaluation of the cytokine profile

On the background of the modeling of endothelial dysfunction by the use of bacterial lipopolysaccharide, the concentrations of TNF-α, IL-6 and IL-10 increased several times and reached 101.88±6.87 PG/ml, 156.93±7.02 PG/ml and 82.78±577 PG/ml, respectively. The correction by tocilizumab resulted in a decrease in the concentration of these cytokines to the intact animals level at the dose of 4 mg/kg and their normalization at the dose of 8 mg/kg.

Fig. 1. The influence of the test compounds on the serum concentration of tumor necrosis factor-α. * – p<0.05 in comparison with the intact animals; ** – p<0.05 in comparison with the control group

Fig. 2. The influence of the test compounds on the serum concentration of interleukin-6. * – p<0.05 in comparison with the intact animals; ** – p<0.05 in comparison with the control group

IL-6 is a multifunctional cytokine that is involved in the pathogenesis of cardiovascular diseases. Increased secretion of IL-6 in response to inflammation, increases serum concentration of angiotensin II, exacerbates oxidative stress and vascular damage that is why IL-6 has become a marker of vascular inflammation, i.e. the increase in the level of circulating interleukin epidemiologically associated with a number of clinically significant diseases of the cardiovascular system [16, 17].

Increased level of circulating IL-6, leads to the following pathological changes, which plays an important role in the development of endothelium-associated pathology:
- activation and maintenance of the low-grade systemic inflammation (including a powerful increase in the synthesis of CRP in a liver);
- disruption of homeostatic functions (cellular protection from reactive oxygen species);
  - endothelial dysfunction,
  - activation of monocytes;
  - intimal proliferation, myocardial hypertrophy;
- disturbance in metabolic control (insulin resistance).

These various effects indicate that IL-6 is not merely a passive biomarker, but actively modulate a course of cardiovascular diseases [18, 19].

In numerous clinical studies there was proved the role of interleukin-6 in the pathogenesis of atherosclerosis [20], ischemic heart disease [16], hypertension [21], chronic heart failure and myocardial hypertrophy [16]. At the same time, there is confirmed and discussed not only the role of the increased level of IL-6 in the development of cardiovascular disease, but adverse effects on the prognosis and outcomes of the disease.

In this regard, IL-6 began to be typified as a potential target for targeted therapy of such diseases as atherosclerosis, coronary heart disease, as well as with the aim of reducing cardiovascular risk with comorbidity.

The most studied pleiotropic effect of tocilizumab is the effect on the atherosclerosis progression. For example, SAMURAI and CHARISMA studies demonstrated the increase in the level of HDL cholesterol in 24% of patients on a background of tocilizumab administration, and in the CHARISMA study there was observed a dose-dependent effect of tocilizumab: the use of the drug in a dose of 8 mg contributed to the increase in the level of HDL cholesterol and reduced the atherogenic index [22, 23].

In experimental studies inhibition of IL-6 by tocilizumab led to improvement in endothelial function and reduced arterial stiffness [24].

Preliminary data obtained in the study of a single dose of tocilizumab in patients with myocardial infarction without ST-segment elevation showed a tendency to decrease in the
areas under the curve of concentrations of CRP and troponin T [25].

In another pilot study, there was investigated the effect of tocilizumab on endothelial dysfunction and arterial wall rigidity. According to the results, tocilizumab significantly improved the endothelium-dependent vasodilation from 3.3 ± 0.8 to 4.4 ± 1.2 (after 3 months of therapy) to 5.2 ± 1.9% (after 6 months of therapy) and decreased the vascular wall stiffness. Thus, the researchers concluded the beneficial effect of tocilizumab on endothelial function and the main pathogenetic links of cardiovascular diseases and complications [26].

The high prevalence of cardiovascular complications in rheumatoid arthritis has led to the need to study the effect of targeted therapy on cardiovascular risk in this group of patients. So, 52 weeks of tocilizumab therapy resulted in marked and significant increase in ejection fraction (+ 8.2%) and a significant decrease in left ventricular mass (-24.4%) [27].

Data review of clinical trials published in the Lancet in 2012, suggests that targeting inhibition of the IL-6 receptors may provide a new therapeutic approach to the prevention and treatment of ischemic heart disease [28].

Dysfunction of endothelium, as a rule, involves in the pathological process not only the cardiovascular system but also leads to the development of the so-called "cardiometabolic continuum". Therefore, it is necessary to consider the potential influence of the IL-6 inhibitors on carbohydrate metabolism and insulin resistance.

The first study of the influence of tocilizumab on carbohydrate metabolism was the study, during which patients with rheumatoid arthritis and coexisting diabetes mellitus type II was treated with tocilizumab at the dose of 8 mg/kg intravenously every 4 weeks [24]. In the subgroup of 10 patients, HbA1c decreased significantly after 1 month tocilizumab therapy (from 7.17 to 6.35%, p <0.01), while the effect was maintained after 6 months (6.0%, p <0.001).

In another study, children with systemic juvenile idiopathic arthritis in the presence of glucocorticoids dosage modification, only after 6 weeks of tocilizumab therapy HOMA-IR index reflecting insulin resistance, significantly decreased [29]. The obtained data were confirmed in another cohort study (n=11): after 3 months of tocilizumab therapy HOMA-IR decreased significantly, indicating improving insulin sensitivity through inhibition of IL-6 signaling. To confirm the theory, LAR was designed as a new marker of insulin resistance. In keeping with HOMA-IR dynamics, LAR was constant during the first month of the study, but it was significantly lower than baseline after 3 months of therapy [30].

Thus, we have described the potential endothelial protective mechanisms of inhibition of signaling pathway triggered by overproduction of interleukin-6, which require further study, both in preclinical and clinical studies.

**Conclusion**

Thus, tocilizumab (at the doses of 4 mg/kg and 8 mg/kg) after a single dose has a pronounced endothelial protective activity in the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress and prevents the imbalance of the cytokine profile.

**Conflicts of interest**

The authors have no conflict of interest to declare.

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