EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Changes in the Concentration of Monocytic Chemotaxic Protein-1 in Patients with Unstable Angina Treated with Arixtra

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The time course of inflammatory reaction markers in the blood of patients with unstable angina was studied during therapy including arixtra. Plasma concentration of monocytic chemotaxic protein-1 (MCP-1) decreased on days 2 and 3 in patients receiving arixtra and a trend to an increase in MCP-1 concentration was observed on day 7 after the drug was discontinued. After 1 month, MCP-1 level decreased in all patients. The concentration of highly sensitive C-reactive protein also decreased 1 month after the disease onset; no changes in the concentrations of IL-8 and IL-2 receptor α-subunit were detected during these periods. It seems that arixtra is characterized by an anti-inflammatory effect manifesting by reduction of plasma chemokine MCP-1 concentration.

Key Words: *unstable angina; monocytic chemotaxic protein-1; chemokines; arixtra*

Inflammatory reaction of the vascular wall is an integral part of the coronary atherosclerotic plaque destabilization. It clinically manifests by development of the acute coronary syndrome. Leukocyte migration from circulating blood to the arterial intima is regulated by chemotaxic cytokines (chemokines), which together with other proinflammatory factors are produced by the vascular wall cells [3]. Monocytic chemotaxic protein-1 (MCP-1) plays the key role in attraction of monocytes to sites of injury/inflammation. High expression of MCP-1 and other cytokines (RANTES, IL-1β, IP-10, MIG, *etc.*) was found in human atherosclerotic plaques [1,11]. The concentrations of MCP-1, IL-8, IL-2 receptor soluble α-subunit (sCD25), IL-6, IL-1β, and other proinflammatory markers are elevated in the blood of patients with unstable angina [2,8,13]. High levels of highly sensitive C-reactive protein (hsCRP) and MCP-1 are independent risk factors for repeated cardiovascular events in patients with a history of acute coronary syndrome (ACS) [5].

Patients with ACS without *ST* segment elevation are treated with anticoagulants based on heparin and its derivatives. In addition to binding the proteolytic enzymes involved in clotting, these compounds are characterized by the direct anti-inflammatory effect, due to binding of cytokines, growth factors, leukocytic adhesion molecules [4,14]. Sodium fondaparinux (arixtra) is a synthetic pentasaccharide, a specific inhibitor of Xa clotting factor. Its efficiency in the treatment of patients with ACS without *ST* segment elevation was proven [9]. According to mass spectrometry data, arixtra is an MCP-1 ligand [15], but its effects on

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blood concentrations of MCP-1 and other cytokines in patients have never been studied.

We studied the time course of chemokines MCP-1 and IL-8, hsCRP, sCD25, IL-1β, and IL-6 in patients with unstable angina receiving standard conservative antianginal therapy including arixtra.

MATERIALS AND METHODS

The study was carried out in 20 patients (13 men and 7 women, mean age 63.9±10.8 years) with ACS without the ECG *ST* segment elevation, in whom subsequent development of myocardial infarction was ruled out by findings of further standard laboratory and clinical instrumental studies and who had no indications for urgent endovascular intervention. Unstable angina was diagnosed from the typical clinical picture (first emerging or progressing angina pectoris), ECG (no ischemic changes or changes in the terminal part of the ventricular complex), negative troponin test or values of this test not reaching the diagnostic criteria of myocardial injury. Patients received standard therapy from the moment of admission to hospital: disaggregants (aspirin, plavix), β-blockers, angiotensin converting enzyme (ACE) inhibitors, statins; 15 patients received sodium fondaparinux anticoagulant (arixtra, GlaxoSmithKline) in a dose of 2.5 mg subcutaneously during 5-6 days.

Measurements of MCP-1, IL-8, hsCRP, sCD25, IL-1β, and IL-6 were carried out on admission, on days 2, 3, 7 and 1 month after admission. Stenting of the symptom-linked vessel was carried out in 10 patients from days 8 to 1 month of hospital treatment. Blood cytokines were analyzed after 1 month only in the patients not subjected to stenting.

Peripheral blood was collected into tubes without anticoagulant and in citrate anticoagulant and centrifuged for 20 min at 2000*g*. Specimens of the serum and citrate-stabilized plasma were stored at -70°C.

Plasma concentration of MCP-1 was measured by EIA using MCP-1 ELISA kit (BioSource).

The concentration of hsCRP was measured by the nephelometric method on a Bering Nephelometer blood protein analyzer (Bering Marburg GmbH) by the standard method.

The levels of IL-6, IL-1β, sCD25, and IL-8 were measured by the chemiluminescent method on an Immulite 1000 analyzer (Siemens) according to the instruction.

The normal distribution of MCP-1 and sCD25 was confirmed by the Kolmogorov–Smirnov and Shapiro–Wilks tests. The significance of differences in the time course of the parameters was evaluated using Student's *t* test for related samples. The distribution of IL-8 and hsCRP levels did not conform to the normal distribution and was evaluated using the Wilcoxon *W* test. The differences were considered statistically significant at $p<0.05$.

RESULTS

Blood level of MCP-1 in patients with unstable angina was 190.1±40.1 pg/ml on day 1, which was in agreement with the data on high MCP-1 concentrations in ACS patients [2,7,10]. The level of MCP-1 gradually decreased, reaching a statistically significant reduction on day 3 in comparison with its initial level (Table 1). On day 7, a trend to elevation of MCP-1 concentration in the blood ($p=0.08$) was noted. We suggested that this elevation could be caused by discontinuation of the anticoagulant, because the rest therapy was not changed. We excluded the patients receiving no anticoagulants from analysis and studied the time course of MCP-1 in 15 patients receiving arixtra.

A significant reduction of MCP-1 level in the blood was seen on days 2 and 3 of arixtra therapy (Fig. 1). A trend to MCP-1 elevation ($p=0.06$) was noted on day

TABLE 1. Cytokine Levels in Patients with Unstable Angina (*M*±*m* or median (percentiles 25-75))

Parameter	Day 1	Day 2	Day 3	Day 7	1 month
MCP-1, pg/ml	190.1±40.1	176.8±37.1	163.9±34.5*	175.3±41.5	164.8±18.8*
hsCRP, mg/liter	1.81	1.36	1.57	1.22	0.677*
	(0.79-6.46)	(0.79-3.35)	(0.83-2.5)	(0.79-1.7)	(0.62-1.04)
IL-8, pg/ml	23.9	18.7	34.6	24.8	27.3
	(13.6-30.0)	(11.7-30.5)	(15.8-74.3)	(15.7-65.5)	(19.0-56.7)
IL-6, pg/ml	3.63	4.89	3.07	4.16	2.31
	(2.0-5.16)	(2.9-5.7)	(2.15-5.21)	(2.12-6.11)	(2.21-3.8)
sCD25, U/ml	474.5±190.2	463.9±174.7	469.8±193.0	488.4±197.3	563.4±219.2

Note. *$p<0.05$ compared to day 1.

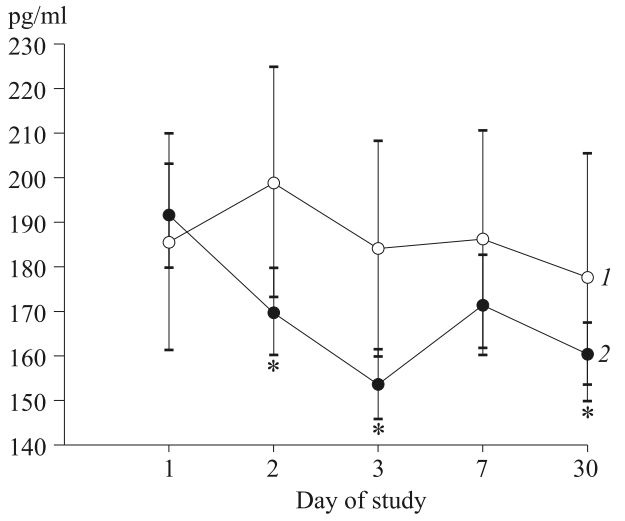

Fig. 1. Time course of MCP-1 concentration in patients with unstable angina in the control (*1*; *n*=5) and during arixtra treatment (*2*; *n*=15). **p*<0.05 compared to the control.

7. No appreciable shifts in MCP-1 level during the first 7 days were found in the patients receiving no arixtra. After 1 month, the blood level of MCP-1 decreased significantly in comparison with its initial level. In order to rule out the impact of arixtra for detection of MCP-1 in plasma samples, arixtra in a concentration of 0.5 mg/liter was added to solutions with the known MCP-1 concentrations and to 5 plasma specimens selected at random and EIA was carried out. According to the known pharmacokinetic characteristics of arixtra, the concentration of 0.5 mg/liter is maximum one after subcutaneous injection of the drug in a dose of 2.5 mg. Addition of arixtra to standard MCP-1 solutions and to plasma specimens caused no changes in the detected MCP-1 levels.

Blood concentrations of hsCRP in patients with unstable angina varied greatly on day 1. A trend to reduction of this parameter was observed during therapy; its level was statistically lower after 1 month of the disease (Table 1). We failed to find statistically significant changes in IL-8 and sCD25 concentrations during the studied periods. The levels of IL-6 and IL-1β surpassing the minimum concentration detectable by the chemiluminescent method (5 and 2 pg/ml) were found in serum specimens of 9 (45%) and 4 (20%) patients, respectively, and hence, the time course of these parameters was not analyzed.

Hence, the level of MCP-1 in the blood gradually decreased in patients with unstable angina during therapy. Presumably, the reduction of MCP-1 level during the first 7 days was due to arixtra therapy and was caused by rapid metabolism of the MCP-1—arixtra complex or by the anti-inflammatory effect of the drug.

The anti-inflammatory effect of arixtra has been demonstrated for the ischemia–reperfusion model on the mouse kidney and rat intestine [12]. The effect manifested by a lesser mortality, reduced concentrations of IL-6 and MIP-2 cytokines in the blood, and expression of these cytokines in involved organs. However, no appreciable effect of arixtra on blood MCP-1 concentration and its expression in tissues was detected on the renal ischemia–reperfusion model [6]. Presumably, this could be due to the fact that single injection of the drug on day 1 after the intervention was used in this study. A delayed (after 1 month) reduction of plasma MCP-1 concentration in our study seemed to be due to stabilization of patient status during the therapy.

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