GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Urokinase Receptors on Human Monocytes in Angina Pectoris

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Expression of urokinase receptors on peripheral human monocytes from healthy donors and patients with angina pectoris is studied by ^{125}I-prourokinase receptor binding assay and flow cytofluorimetry with monoclonal antibodies to the urokinase receptor. Plasma concentration of urokinase is measured by ELISA. It is shown that in patients with angina pectoris plasma content of urokinase is higher by 30%, while the number of free urokinase receptors on monocytes is half lower than that in healthy donors.

Key Words: *monocytes; angina pectoris; urokinase; urokinase receptor*

Coronary heart disease is associated with atherosclerotic processes in coronary vessels. Proper functioning of the fibrinolytic plasmin/plasminogen activator system is an important mechanism of thrombosis protection. Activity of plasminogen activator in human vessels involved into atherosclerotic process is reduced in comparison with intact vessels [7].

Urokinase plasminogen activator (urokinase, UR) is produced by activated monocytes, endotheliocytes and vascular smooth muscle cells [2]. These cells also possess urokinase receptors (URR). Urokinase is primarily associated with cells, and only low UR concentration is present in the plasma [4]. Expression of URR dramatically increases in arterial lesions, atheromas, and atherosclerotic plaques [8,10]. Urokinase has a catalytic domain (serine protease) and growth factor-like domain interacting with specific receptors. Binding of UR to URR is accompanied by a considerable increase in proteolytic activity of its catalytic domain [6]. There are usually two pools of URR in peripheral monocytes: free and occupied by endogenous UR [6]. Receptor-bound UR degrades the extracellular matrix thus promoting monocyte migration into the vascular wall in response to chemotactic and activating stimuli. Inflammation cytokines (interferon-γ and tumor necrosis factor-α) manyfold increase URR expression in monocytes [5]. Monocytes/macrophages participate in all stages of atherogenesis [14] and, being producents of a variety of bioactive substances, are widely used as markers of inflammation in coronary vessels and in angina pectoris [9]. The aim of the present study was to investigate the expression of URR in monocytes and to measure plasma content of UR in patients with angina pectoris.

MATERIALS AND METHODS

Experiments were carried out on peripheral monocytes and blood plasma from 10 healthy donors and 17 patients with angina pectoris. All patients were examined in accordance with the Cardiology Center Ethical Committee protocol and had coronary heart

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TABLE 1. Expression of URR on Monocytes from Healthy Donors and Patients with Angina Pectoris (*M±m*)

Group	Receptor binding, number of receptors per cell	Cytofluorimetry, % of fluorescence intensity
Healthy donors	10770±2490 (5)	90±5 (10)
Patients with angina pectoris	4810±1220 (8)	72±23 (13)

Note. Number of donors and patients is shown in parentheses.

disease — stable angina functional class III and angiographically proven coronary atherosclerosis. The patients received standard therapy, which included aspirin, β-blocker or calcium antagonists, and hypolipidemic agents (lovastatin or simvastatin). Mononuclear leukocytes were isolated using a modified method [1]. Monocytes were isolated by adhesion to plastic in RPMI-1640 medium containing 5% bovine embryonic serum in a CO_2-incubator at 37°C for 1 h. The number of free saturable URR on monocytes was determined using ^{125}I-labeled inactive single-chain pro-UR (Laboratory of Gene Engineering, Russian Cardiology Research-and-Manufacturing Complex). Pro-UR was labeled as described previously [5]; specific radioactivity of the preparation was 1-2.5×10^6 dpm/pmol UR. After adhesion to 24-well plates the cells were washed, and binding with ^{125}I-pro-UR was carried out at 4°C for 16 h [5,11]. Nonspecific binding was measured in the presence of a 100-fold excess of cold pro-UR. Radioactivity was measured in a Compu Gamma γ-counter (LKB). The number of URR and their K_d was calculated from Scatchard plots.

Expression of URR on monocytes was analyzed by indirect immunofluorescence in a flow cytofluorimeter (FACScan, Bekton Dickinson). Erythrocytes were removed by lysis in the presence of NH_4Cl; the cells were washed with phosphate buffer containing 1% bovine serum albumin and incubated with mouse monoclonal antibodies to URR (American Diagnostica Inc.) at 4°C for 30 min. These antibodies bind to both free and occupied by endogenous UR receptors. After washout, the cells were incubated with rabbit antimouse immunoglobulin antibodies under the same conditions and fixed in 1% paraformaldehyde. The data were expressed in relative fluorescence units.

Plasma content of UR was measured by enzyme-linked immunosorbent assay using UMUBIND uPA ELISA kits (American Diagnostica Inc.), which allowed us to detect both high- and low-molecular-weight UR, as well as UR bound to receptors and plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2). Urine active UR was used as the standard. Absorbance was measured at 492 nm in a Titertek Multiscan MC multichannel spectrophotometer (Labsystems).

The data were processed statistically using the Student *t* test.

RESULTS

The numbers of URR in monocytes from healthy donors and patients with stable angina are presented in Table 1. The number of free URR (binding of ^{125}I-proUR) varied from 6100 to 17,460 in healthy donors and from 1570 to 100,680 in patients, K_d being 0.5-1.5 nM in both groups. Thus, expression of URR in monocytes from patients with stable angina was 2.2-fold lower than in cells from healthy donors ($p<0.05$).

Plasma content of UR in patients surpassed that in donors by 33.5%: 10.47±3.17 (from 4.64 to 15.45, $n=15$) in *vs.* 7.84±2.15 nM (from 5.18 to 10.82, $n=10$). Thus, the number of free URR in peripheral monocytes from patients with stable angina is reduced.

It can be assumed that plasma UR is elevated due to complexation with PAI-1. The concentration of PAI-1 is increased in coronary atherosclerosis [12]. Previous data [13] suggest that URR persists not only in a glycosylphosphatidylinositol-anchored form in cells but also in a soluble form in the plasma. The formation of the URR—UR—PAI—1 complex in the plasma can be hypothesized, which can be detected by the used ELISA.

Reduction in the number of URR on monocytes can be due to internalization of the receptors, which requires formation of the URR—UR—PAI—1 complex on cell surface [3]. The increase in the plasma content of PAI-1 probably stimulates this complexation and internalization of formed complexes and leads to a decrease in the number of URR.

Expression of URR on monocytes is responsible for proteolytic activity of these cells, so that monocytes in the thrombus promote fibrinolysis. It can be assumed that the decreased number of free URR on monocytes in angina pectoris in partially responsible for the reduced fibrinolytic activity in these patients.

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Effect of Antiserotonin Antibodies on Functional Activity of T and B Lymphocytes and Peritoneal Macrophages

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Experiments on C57Bl/6 mice showed that antiserotonin antibodies injected intraperitoneally in a dose of 25 mg/kg or added to cell culture in a dose of 10^{-7} mol/ml suppress lymphocyte proliferative response to pokeweed mitogen and stimulate functional activity of macrophages.

Key Words: *antiserotonin antibodies; T lymphocytes; B lymphocytes; peritoneal macrophages*

Both neurotransmitters and neuropeptides are involved in neuroimmune interrelationships and interactions. Neuroimmunomodulatory function of serotonin (5-HT) has been demonstrated [3,4]. It was shown that activation of serotoninergic mechanisms in the central nervous system is accompanied by suppression of the immune system mediated through pituitary-adrenal regulatory mechanisms. The possibility of direct effect of 5-HT or antiserotonin antibodies (ASAB) on immunocytes has not yet been explored. Previously, we showed that 5-HT and ASAB stimulate functional activity of macrophages [5]. The aim of the present study was to compare the immunomodulating effect of ASAB on functional activity of macrophages and lymphocytes.

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MATERIALS AND METHODS

Experiments were carried out on C57Bl/6 male mice weighing 23-24 g. In series I the effect of systemic injection of ASAB on functional activity of T and B lymphocytes and peritoneal macrophages was studied. Antiserotonin antibodies were injected intraperitoneally in a single dose of 25 mg/kg (protein concentration). In series II we studied the effect of ASAB (10^{-7}mol/ml) on the same immunological parameters in cell culture.

Antiserotonin antibodies were isolated from rabbits immunized according to the standard scheme [1]

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