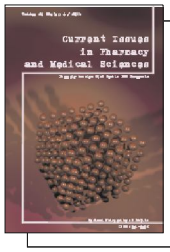

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Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Arginase/nitric oxide synthase regulatory mechanism of cells under the action of trigger factors that cause reactive arthritis

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ABSTRACT

On the basis of the research it can be argued that the determination of the enzymatic activity of arginase and NO-synthase of peripheral blood lymphocytes gives a qualitative informative assessment on the functioning of the immune cells, as changes in the activity of arginase reflect the NO metabolism. In the research, the optimal conditions of arginase reaction were determined and some of kinetic parameters of arginase were established. The significant growth of arginase activity (3.3 times) and iNO-synthase activity (3.8 times) in peripheral blood lymphocytes in patients with reactive arthritis compared with practically healthy donors was shown.

Keywords: arginase, L-arginine, NO-synthase, reactive arthritis, lymphocytes

INTRODUCTION

Reactive arthritis (ReA) is a systemic joint damage that develops as a result of chlamydia or, rarely, other infection [2, 5]. It is accompanied by nonsuppurative inflammation of the joints as a result of immune disorders caused by various types of infection [2, 4, 5].

In the pathogenesis of ReA central role belongs to immune disorders, particularly related to the functioning of T-lymphocytes [2], as well as metabolic disturbances [13]. During the last decade, considerable attention has been paid to the arginine metabolism and the role of nitric oxide (NO) in the pathogenesis of rheumatic diseases [1]. Significance of L-arginine increased sharply after proving that it is a precursor of NO, which has a wide range of bioregulatory effects [4, 9]. Infectious factors cause immune stresses, due to the action of cytokines, and stimulate synthesis of NO in patients with arthritis [1, 11, 15]. Under these conditions, NO contributes to immune defence of the organism, acting as immunoregulator, and it also exhibits cytotoxic effect in high concentrations that complicates various signs of autoimmune nature [14]. It should be noted that formation of NO, precursor of which is amino acid L-arginine, is not associated only with synthetic pathway of metabolism. L-arginine metabolism takes place by at least in two ways: oxidative – (NO-synthase) and nonoxidative (arginase) [6, 9]. It is important to emphasize, that the NO-synthase and arginase can compete for the substrate – L-arginine. Arginase is a metaloenzyme that catalyzes the hydrolytic cleavage of L-arginine to urea and L-ornithine. Arginase regulates the formation of NO by competing with NO synthase for L-arginine [9]. The physiological role of arginase due to its participation in numerous metabolic processes in the cell indicates that the enzyme belongs to the important link in the development of many pathological conditions of the organism, including autoimmune diseases [9].

The aim of the study was to investigate the activity of arginase and NO-synthase in peripheral blood lymphocytes in patients with reactive arthritis.

MATERIALS AND METHODS

The peripheral blood lymphocytes were isolated from patients (n=21) who were hospitalized in the Rheumatology Department of the Lviv Regional Hospital. For an objective clinical evaluation of the initial patients' condi-

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DOI: 10.12923/j.2084-980X/26.4/a.06

tion and for the effectiveness of the conducted treatment, the diagnosis was made on the basis of uniform diagnostic criteria, approved at a joint Plenum of rheumatologists and orthopedic-traumatologists of Ukraine (2003). The control group consisted of practically (clinically) healthy donors at the age of 20–30 years (n=16). Mononuclear peripheral blood lymphocytes of the patients and healthy persons were isolated from heparinized recently-received venous blood in the gradient of *ficoll-triombrast* density (r=1.077–1.090) [3]. The cells stained with 0.1% trypan blue were counted in the Goryaev's camera. Integrity and viability of lymphocytes was assessed by trypan blue staining. In all experiments, it was at least 95%. Enzymatic activity of the arginase was determined in the permeabilized peripheral blood lymphocytes. For permeabilization of the membranes to suspension of the lymphocytes 0.1% saponin was added. Assay of the arginase activity was carried out by the formation of the urea, which concentration was determined using diagnostic kit according to the instructions of the manufacturer (Simko, Ukraine). Enzymatic reaction was initiated by addition of saponin permeabilized lymphocytes aliquots into the reaction mixture of the following composition: 20 mM tris-sulfate buffer, pH 9.5, containing 2 mM MnCl2 and 100 mM arginine in a final volume of 1 ml. The protein amount in the sample did not exceed 50-100 mkg/ml. After the incubation for 15–30 min at 37°C, the resulting urea was assayed spectrophotometrically at 520 nm. One unit of enzyme activity was defined as the amount of enzyme that released 1.0 μmol of urea for 1 min under the given conditions. The protein concentration in the lymphocyte mixture was determined by Lowry method [8]. The reaction was stopped by addition into the reaction mixture 50% trichloroacetic acid (TCA). In the control samples, the appropriate aliquot of the saline solution, instead of lymphocyte suspension, was added. The activity of the endothelial (eNO) and inducible (iNO)-synthases was determined using the method described [1]. The calculations were carried out using the software MS Office. The results were treated with conventional methods of the variation statistics using Student's criteria.

RESULTS AND DISCUSSION

Since peripheral blood lymphocytes are the key cells of the immune system and play an essential role in providing compensatory-adaptive reactions of the organism, it is likely, they can be a model for studying of basic metabolic changes that occur in the body at rheumatic pathology. In order to study the characteristics and mechanism of the arginase functioning in blood lymphocytes, some of its kinetic parameters were determined. The lymphocyte suspension was incubated in the reaction mixture during different periods. These experiments showed that the kinetic of the arginase reaction in the saponin-permeabilized lymphocytes expressed by the curve that tends to saturation.

In the lymphocytes of healthy donors arginase activity was 106.0 ± 6.7 while in the lymphocytes of patients with ReA – 349.8 ± 17.2 nmol urea/min·mg of protein (Table 1).

Table 1. Comparison of arginase and NOS activities in lymphocytes of patients with ReA and healthy donors (control)

Enzymes	Control	ReA
Arginase, nmol urea/min·mg of protein	106±6.7	349.8±17.2
eNOS, nmol NADPH(H^+)/min·mg of protein	74.6±6.3	39.7±3.2
iNOS, nmol NADPH(H^+)/min·mg of protein	–	152.3±14.4

The dependence of arginase activity from the concentration of the substrate in peripheral blood lymphocytes was studied. L-Arginine was added to the reaction mixture in the concentrations from 1 to 200 mM (at constant concentration of Mn^{2+}, 2 mM). Thus, there is a linear increase of arginase activity followed by the plateau. Throughout the range of the L-arginine concentrations, arginase activity of the patients with ReA is increased, comparing with the healthy donors. The highest activity of the studied enzyme is observed in the presence of 150 mM of L-arginine in the reaction mixture. The maximum amount of the reaction product of healthy persons and patients with ReA differs significantly. Based on studied kinetic parameters, we have assumed that in peripheral blood lymphocytes of patients with ReA accumulation of urea is faster and more active, comparing with healthy donors.

Since the metabolism of the L-arginine depends on the activity of the NO-synthases, activity of the eNO-synthase and iNO-synthase were determined. It was found, that the activity of NO-synthase in the saponin permeabilized lymphocytes of the healthy persons is 74.6±6.34 nmol NADH(H^+) / min·mg of protein (n = 14). Given that iNO-synthase in healthy person is absent, we can assume that this quantity is the activity of eNO-synthase. A significant decrease of eNO-synthase in the lymphocytes of the patients with ReA, that differs in 1.88 times from healthy persons was shown. At the same time, the iNO-synthase is activated and in the lymphocytes of patients with ReA increases to 152.3±14.4 nmol NADH(H^+)/min·mg of protein (n=16).

Recent studies showed that the immunopathological processes depend on the level of NO oxide in the organism. Increase of arginase and iNO-synthase activities of lymphocytes indicate changes in functional activity of immunocompetent cells, which may be caused by metabolism processes disorder in these cells, and may also mediate through other regulatory mechanisms of the cell (ions Ca^{2+}, NO). There are also other data about the increasing

of the arginase activity in the pathological conditions. The high arginase activity level of peripheral blood mononuclear cells in HIV positive patients was shown [4]. Increase of arginase activity of mononuclear cells after traumatic states is observed [10].

The increase of arginase activity level in blood plasma was set only in patients with rheumatoid arthritis, whereas in patients with systemic lupus erythematosus and osteoarthritis no significant changes were observed [7]. Increased activity of arginase in macrophages isolated from synovial fluid of patients with rheumatoid arthritis was shown [5, 12].

CONCLUSIONS

In the research, the optimal conditions of arginase reaction were determined and some of kinetic parameters of arginase were studied. The significant growth of arginase activity and iNO-synthase activity in peripheral blood lymphocytes in patients with reactive arthritis compared with practically healthy donors was shown.

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