DETECTION OF XANTHINE OXIDASE ACTIVITY IN HUMAN BLOOD MONONUCLEARS

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Xanthine oxidase (XO) is a molybdenum-, iron-, and copper-containing flavoprotein which catalyzes in man the terminal stage of purine oxidation and also the oxidative transformation of pteridines and certain aliphatic and aromatic aldehydes [10]. The greatest XO activity in man is found in tissues of the liver and intestinal mucosa [9]. Leukocytes, including human blood mononuclears, according to the results of spectrophotometric [4], radiometric [11], and cytochemical [3] investigations, do not contain XO activity in solubilized form. On this basis, in the overwhelming majority of cases it has been concluded that the enzyme is not present in these cells, as would seem to be confirmed by the results of immunohistochemical analysis also [7, 8]. However, there is abundant indirect evidence, including functional changes developing in lymphocytes under the influence of activators and inhibitors of XO [2], that human lymphoid cells may contain this enzyme, in a membrane-associated form for example.

It was accordingly decided to investigate whether xanthine oxidase activity is present in a lysate of human blood mononuclears after preliminary solubilization.

EXPERIMENTAL METHOD

Mononuclear cells were obtained by isopycnic centrifugation (800g, 45 min) of venous blood from 24 healthy individuals, stabilized with heparin (Gedeon Richter, 25 U/ml), in a Ficoll – Isopaque density gradient (ρ = 1.077 g/ml). The isolated cells were washed in medium 199 and their viability tested with trypan blue. The mononuclear lysate was obtained by freezing and thawing 3 times in the presence of 0.1% Triton X-100 solution.

Xanthine oxidase activity was determined by Battelli's method [5] in our own modification. The incubation medium (60 min, 37°C) contained (all from Serva): xanthine 100 μM, NAD 66 μM, pyruvate 50 μM, lactate dehydrogenase 150 ncat. The reaction was stopped by cooling the samples on an ice bath. The optical density was measured at 290 nm on a DU-50 spectrophotometer (Beckman).

To verify the specificity of the reaction, a stage of preincubation of the lysate was introduced with the XO inhibitors allopurinol (10^{-4} M) and folic acid (10^{-4} M) for 20 min. The results were expressed in μmoles uric acid/g protein·min (units). The protein concentration in the samples was determined by Bradford's method [6]. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney U test.

EXPERIMENTAL RESULTS

Blood mononuclears exhibit XO activity amounting to 2.76 ± 0.029 units. These values are quite low and correspond to about 1% of activity recorded in the liver and about 0.4% of XO activity in the mucous membrane of the small intestine. The action of XO inhibitors is accompanied by a decrease in activity of the enzyme by 71.4 ± 6.1% if allopurinol

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is used ($p < 0.01$) and by 78.3 ± 7.4% if folic acid is used ($p < 0.01$). The presence of the enzyme in mononuclears in the membrane-associated form explains the negative results of investigations cited above, in which attempts were made to detect XO activity in the soluble fraction of cell material. Our own data are to some degree contradictory to the results of immunohistochemical investigations [8], during which XO was not identified as an immunoreactive protein in human leukocytes. However, if it is recalled that as the immunizing material the authors cited above used the enzyme from cows' milk, it can be postulated that the XO antibodies obtained did not possess sufficient cross-immunoreactivity with the enzyme in human leukocytes. On the other hand, detection of XO activity in lymphoid cells may provide an explanation, for example, of the as yet unexplained mechanism of the immunomodulating effect of adenosine and inosine [1], which are converted in the human body in the course of a few minutes into hypoxanthine and xanthine, which are substrates of XO. This state of affairs is evidence that besides the enzymes of the initial stages of purine catabolism (adenosine deaminase, purine-nucleoside phosphorylase), whose importance in the immune response is well known, the enzyme of the terminal stage of purine breakdown, namely XO, probably plays an essential role in maintenance of the functional activity of the lymphocytes. Another illustration of the importance of the role of XO in the above-mentioned processes is given by the results of our previous studies, according to which the lymphocytes of patients infected with HIV (and not yet exhibiting the clinical features of AIDS), already show a significant (by 62.2%) increase in XO activity. In this connection there is reason to suppose that the terminal component of purine catabolism in lymphocytes, i.e., xanthine oxidase, will be the next molecular target for a new class of immunocorrectors.

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