PHARMACOLOGY

Comparative Study of Purine Antagonist Interactions with Blood Lymphocyte and Bone Marrow Lymphoblast Adenosine Receptors

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Azathioprine (AZT) is the most widely used of the 6-mercaptopurine (6-MP) analogs belonging to the purine antagonist immunosuppressives. A direct effect of these drugs on DNA synthesis in lymphoid cells underlies their wide use in the therapy of acute lymphoblastic leukemia and various autoimmune diseases. The putative mechanisms of the cytotoxic effect of 6-MP are its chemical transformation into a nucleotide [1], followed by a feedback inhibition of endogenous purine intracellular synthesis [6], and 6-MP incorporation in nucleic acids, causing structural disturbances [2]. The mechanism of purine antagonist selective action on lymphoid cells is still to be researched [2], as is the cause of the differing sensitivity of tumor cells to these drugs.

Purine adenosine receptors are known to be represented in many lymphoid cellular types, namely, lymphocytes, mononuclear phagocytes [10], and granulocytes [11].

There is some evidence of the participation of purine receptor endogenous and exogenous ligands in lymphopoiesis regulation [9]. Reduced activity of adenosine deaminase, one of the enzymes of purine metabolism, leading to an increase of the lymphocyte adenosine content, is the cause of congenital immunodeficiency [4].

The aim of the present research was to investigate the role of the purinergic receptor component in the mechanism of 6-MP and azathioprine interaction with blood lymphocytes and bone marrow lymphoblasts.

One of the tasks of this study was to compare ^{3}H-NECA (an A_2-purine receptor selective ligand) specific binding to blood lymphocytes and bone marrow lymphoblasts and to determine the relative affinity of 6-MP and AZT to the purine receptors of these cells.

MATERIALS AND METHODS

Bone marrow blast cells obtained from patients with acute lymphoblastic leukemia were used in the study. Approximately 0.5 ml of puncture biopsy specimen was taken in a tube and washed twice in HEPES buffer at pH 7.35 [3].

A peripheral blood mononuclear suspension was prepared after Boyum (1968).

To samples each containing 120 μl of cell suspension (the final concentration being $10\text{-}20\times10^6$

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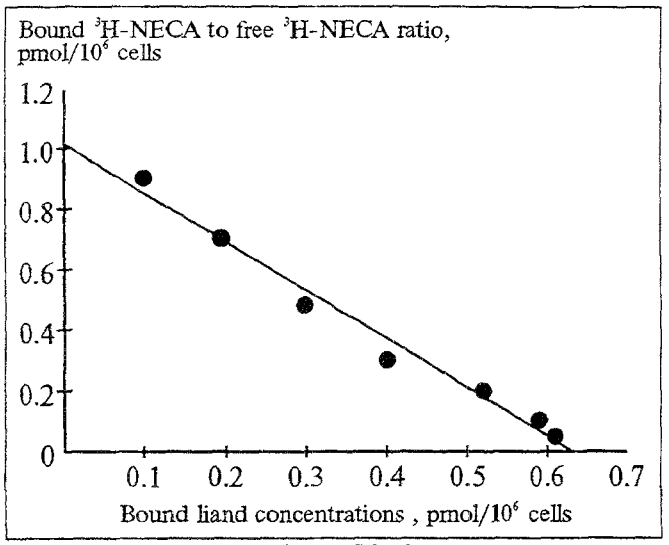

Fig. 1. Specific binding of ^{3}H–NECA by lymphocytes, represented in Scatchard coordinates.

cells/ml) 10 µl of labeled NECA (Amersham) solution in HEPES buffer with final concentrations of 0.1-5 µM and 10 µl unlabeled adenosine (Serva) excess were added. The final concentration of adenosine in the incubation medium was 1×10^{-6} to 3×10^{-5} M. The samples were incubated for 30 min at 37°C. Specific ^{3}H-NECA binding was calculated as the difference between the amount of cell-bound NECA in the absence and presence of unlabeled adenosine.

Concurrent analysis of ^{3}H-NECA cell binding in the presence of immunosuppressives was carried out in accordance with the above scheme. The final concentrations of 6-MP (Jarma) and AZT (Serva) in the incubation media were 1×10^{-6} to 3×10^{-5} M.

After incubation, sample aliquots (100 µl) were rapidly pipetted on GF/C (Whatman) filters and washed twice in 2 ml of HEPES buffer at 4°C. The filters were dried at room temperature and placed in flasks with scintillation liquid for the measurement of labeled NECA content by liquid radiometry.

The Scatchard transform was used to mathematically describe the kinetics of substance-receptor interactions [8]. The confidence intervals of the experimental values were calculated and the reliability of the differences between them was assessed using Student's method with p=0.05 [5] and standard EPSON PC J2 computer software.

RESULTS

The analysis of NECA specific binding by lymphocytes in Scatchard coordinates is shown in Fig. 1. The diagram is described by the eduation $Y = 1.1 - 1.75X$. The rectilinear dependence indicates the presence of one type of ^{3}H-NECA binding sites on the lymphocyte membrane, characterized by the following parameters: equilibrium dissociation constant (K_d) is 0.57±0.19 µM, binding site concentration (B_{max}) is 0.63±0.18 pmol/10^6 cells or 360,000 binding sites per cell on the average.

Using concurrent analysis, the adenosine, 6-MP, and AZT concentrations were measured in which ^{3}H-NECA specific binding constituted 50% of the initial value (IC_{50}). For adenosine IC_{50} was 9×10^{-7} M, for AZT 2×10^{-5} M, and for 6-MP 8×10^{-5} M.

These results permitted the conclusion that therapeutic concentrations of AZT [7] but not 6-MP were able to interact with human peripheral blood lymphocyte purine receptors of type A_2.

The Scatchard diagram characterizing ^{3}H-NECA binding by lymphoblasts is concave (Fig. 2) and is made up of two straight lines. Such a diagram shape suggests two types of ligand binding sites, as was confirmed by subsequent studies using a 100-fold AZT and 6-MP excess.

Thus, in contrast to lymphocytes, bone marrow lymphoblasts have two types of ^{3}H-NECA binding sites. Type I sites are high-affinity, characterized by K_d = 0.53±0.15 µM and maximal binding capacity B_{max} = 0.25 pmol/10^6 cells; type II sites are low-affinity, with K_d = 6.5±1.0 µM and B_{max} = 1.5±0.2 pmol/10^6 cells. The number of labeled NECA high-affinity binding sites on lymphoblasts is 50-70% lower on average than on lymphocytes. The ratio of the two types of receptor sites differs significantly in individual cases.

The descending rank of the relative affinity of the studied compounds to the A_2 receptor second subtype is as follows: 6-MP > adenosine > AZT. For NECA IC_{50} is 4.2×10^{-5} M, for adenosine 6.6×10^{-6} M, for 6-MP 1.3×10^{-6} M, for AZT more than

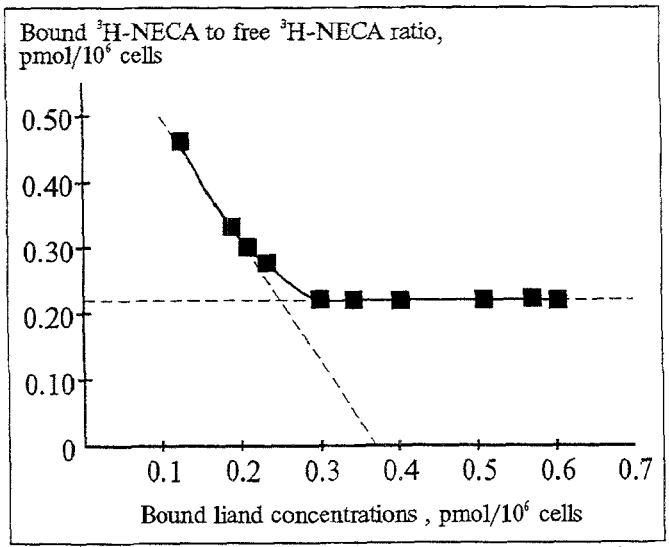

Fig. 2. Specific binding of ^{3}H–NECA by bone marrow lymphoblasts in Scatchard coordinates. Specific binding was assessed in the presence of 100-fold adenosine excess.

6.0×10^{-5} M. Hence, ^{3}H-NECA low-affinity binding sites are targets of 6-MP cytostatic action.

The experimental findings indicate that the selective sensitivity of bone marrow lymphoblasts to 6-MP depends on the presence of specific binding sites of the cytostatic on the surface of these cells. At the same time, azathioprine, a chemical analog of 6-MP, metabolized in the body to 6-MP [4], may directly interact with peripheral blood lymphocyte purine receptors and thus regulate their functional activity.

REFERENCES

1. G. K. Gerasimova, L. V. Matveev, T. A. Sidorova, and O. D. Golenko, *Eksp. Onkol.*, № 4, 29-33 (1982).
2. G. K. Gerasimova, T. A. Sidorova, M. A. Volkova, *et al.*, *Vest. Akad. Nauk SSSR*, № 5, 78-85 (1984).
3. E. S. Gershtein, K. D. Smirnova, and L. S. Bassalyk, *Eksp. Onkol.*, № 2, 33-35 (1990).
4. *Clinical Immunology and Allergology*, Ed. C. Steffen and H. Ludwig, Elsevier-North Holland (1981).
5. G. F. Lakin, Biometry [in Russian], Moscow (1990).
6. Yu. K. Napolov and K. B. Borisov, *Farmakol. Toksikol.*, № 4, 80-87 (1991).
7. A. G. Rumyantsev, *Pediatriya*, №5, 57-62 (1980).
8. P. V. Sergeev and N. L. Shimanovskii, Receptors [in Russian], Moscow (1987).
9. J. S. Bonnafous, J. Dornand, J. Faverd, and J. S. Mani, *J. Recept. Res.*, **2**, № 4, 347-366 (1981-1982).
10. D. Lappin and K. Whaley, *Clin. Exp. Immunol.*, **57**, № 2, 454-460 (1984).
11. G. Marone, S. Vigorita, G. Antonelli, *et al.*, *Life Sci.*, **36**, № 4, 339-345 (1985).

Bioenergetic Mechanisms of the Antihypoxic Effect of Mexidol, a Succinate-Containing Derivative of 3-Hydroxypyridine

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One of the priority research trends at present in the creation of antihypoxic agents is an attempt to activate in hypoxia the compensatory metabolic streams supplying energy substrates to the respiratory system and acting as emergency adaptation mechanisms in this pathological process. The succinate oxidase oxidation pathway in the mitochondria is one of these streams [2,3]. Since the direct administration of succinate as an antihypoxic agent is ineffective because of its relatively poor penetration through the tissue-blood barriers, attempts are being made to use for this purpose various organic compounds containing succinic acid and facilitating its transfer across the cell membrane [1]. We have demonstrated the antihypoxic effect of mexidol, a succinate-containing 3-hydroxypyridine derivative whose properties are manifested predominantly in rats liable to hypoxia [5] and in the myocardium of such animals.

The present research was aimed at studying the bioenergetic effects of mexidol in hypoxia and at proving that succinate oxidation underlies the antihypoxic effect of the drug, succinate being a component of hydroxypyridine, which acting as an energy

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