Effect of Pyrimidine Derivatives on Adenylate Cyclase System of Immunocompetent Cell Regulation *In Vitro*

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The pyrimidine derivatives xymedone and diucifon decrease the activity of adenylate cyclase, as shown in experiments on thymocytes and lymphocytes of guinea pig lymph nodes and human peripheral blood lymphocytes. Inhibition of the enzyme depends on the subpopulation and species appurtenance of immunocompetent cells. The relationship between the results and effects of pyrimidine derivatives in experiment and clinical setting is discussed.

Key Words: *adenylate cyclase; xymedone; diucifon; immunocompetent cells*

Molecular mechanisms regulating the activity of immunocompetent cells and of known drugs have been extensively investigated in modern immunopharmacology. The immunoregulatory effect of hormones and cytokines is mediated by second messengers [3]. These versatile effector systems may possess no tissue and species specificity [1,12]. Two systems of external signal transfer into a cell were studied in sufficient detail: adenylate cyclase and Ca^{2+}-polyphosphoinositol [1,4].

Immunocompetent cells are an adequate model for analysis of intracellular effector systems [5,6]. Adenylate cyclase (AC) of these cells is regarded as a well-reproducible test system for drug screening [2]. Previously, we showed that pyrimidine derivatives are characterized by a total-system immunomodulating action, which suggests realization of their immunotropic effect through universal mechanisms of intracellular regulation.

Our purpose was to study the effects of the pyrimidine drugs xymedone and diucifon on AC activity in immunocompetent cells.

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MATERIALS AND METHODS

Guinea pig thymocytes and lymph node cells (from 15 animals) and human peripheral blood lymphocytes (from 10 subjects) were studied *in vitro*. Guinea pig thymocytes and lymph node cells were isolated from homogenates, and human peripheral blood lymphocytes by centrifugation in a Ficoll-Verograffin density gradient. Cells were resuspended in medium 199 (pH 7.4). AC activity was measured as described previously [15] using α-^{32}P-ATP. Reaction medium (final volume 400 μl) contained 20 μl cell suspension (2×10^6 cell/ml), 1 μCi α-^{32}P-ATP, 50 mM Tris-HCl (pH 7.4 at 37°C), 5 mM $MgCl_2$, 1 mM cAMP, 1 mM EDTA, 1 mM dithiothreitol, 5 mM creatine phosphate, 0.5 mg/ml creatine phosphokinase, 5×10^{-5} M ATP, 0.2% bovine serum albumin, 10^{-5} GTP, and test preparation in concentrations 10^{-3} or 10^{-4} M. Cells were incubated for 30 min at 37°C. The reaction was stopped by adding 200 μl 0.5 N HCl, after which the samples were immersed in boiling water for 6 min at 100°C. Imidasole (1.5 M, 200 μl) was added. The samples were layered onto a column with Al_2O_3 (neutral according to Brockmann II) and eluted with bidistilled water. Radioactivity was measured in a Delta-300 scintillation radiometer. AC activity was expressed in pmol ^{32}P-cAMP/mg pro-

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tein/min. Protein was measured in trichloroacetic precipitate [11]. Each concentration was tested 3 times. Chemical formula of test compounds were as follows: xymedone, 1,2-dihydro-4,6-dimethyl-N-(β-hydroxyethyl)pyrimidone-2 and diucifon, para-para'-(2,4-dioxo-1,3-dihydro-6-methylpyrimidinyl-5-sulfonamino)diphenylsulfone. Results were statistically processed using Student's *t* test.

RESULTS

Basal activity of AC in immunocompetent cells of guinea pigs depends on cell phenotype, which is in line with previous reports [12] and is confirmed by time fluctuations. Therefore, we had an independent internal control for each experiment. Xymedone in concentrations 10^{-4} and 10^{-3} M significantly decreased the activity of AC both in thymocytes and lymph node lymphocytes ($p<0.05$, Fig. 1). AC regulation of guinea pig thymocytes and lymphocytes demonstrated a compatible sensitivity to xymedone. Diucifon did not notably change the activity of guinea pig thymocytes when added in a concentration of 10^{-3} M.

Both xymedone and diucifon decreased AC activity in human peripheral blood lymphocytes in both concentrations ($p<0.05$, Fig. 2). The decrease in AC activity induced by xymedone did not depend on the drug concentration in the incubation medium. The effect of diucifon was stronger than that of xymedone and was dose-dependent. The maximum decrease in AC activity was observed at a diucifon concentration of 10^{-3} M.

Adenylate cyclase is present in high amounts in T and B lymphocyte membranes [10,13]; both stimulation and inhibition of the enzyme activity are regulated [9]. Agonists of muscarinic, α-adrenergic, opiate, and serotonin (5-HT_2) receptors and antagonists of H_2-receptors inhibit AC. Despite some discrepancies in the findings, the majority of researchers agree that a high level of intracellular cAMP suppresses the proliferation of both T and B lymphocytes [7,10]. It is obvious that xymedone-induced decrease in immunocompetent cell AC activity during the early stages is associated with a drop in the intracellular cAMP level. This property of the drug agrees with its activation of lymphocyte proliferation in other test systems. The proliferation phenomenon is conjugated with activation of cellular production of RNA and DNA. Since stimulation of RNA and DNA production is blocked by increased concentration of intracellular cAMP, the decrease in AC activity induced by xymedone gives grounds for initiation of cell proliferation.

Xymedone stimulates erythropoiesis *in vivo*. Bone marrow differentiation of erythroid cells is associated with a continuous progressive drop in basal AC activity [14].

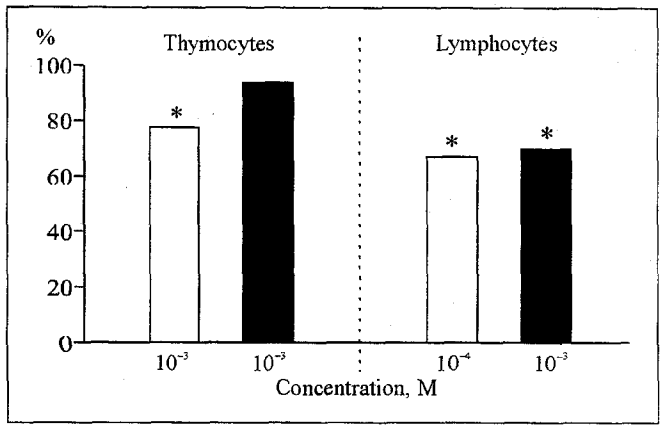

Fig. 1. Adenylate cyclase activity in guinea pig thymocytes and lymph node lymphocytes under the effect of xymedone (light bars) and diucifon (dark bars) in different concentrations. Here and in Fig. 2: *$p<0.05$ *vs.* the control (100%).

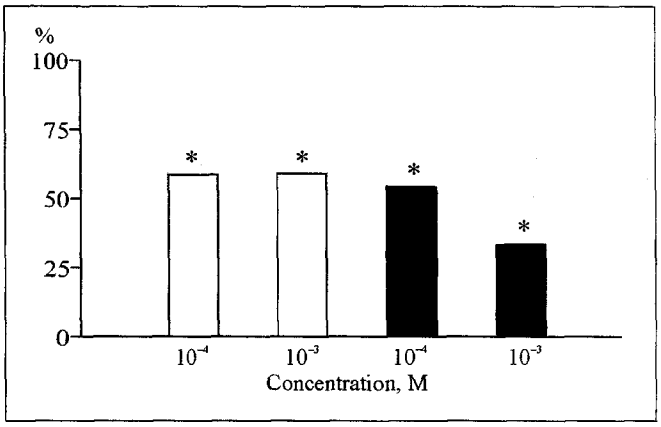

Fig. 2. Adenylate cyclase activity of human peripheral blood lymphocytes under the effect of xymedone (light bars) and diucifon (dark bars) in different concentrations.

Recent studies revealed that compounds increasing the intracellular cAMP content enhance IgE production by stimulating the switch-over of expressed isotope to IgE heavy chain locus [8]. On the other hand, xymedone suppresses IgE production in patients with atopic diseases.

Based on published data and our findings, we have concluded that the decrease in AC activity is one of molecular mechanisms initiating cellular production of nucleic acids and cell proliferation and differentiation under the effect of pyrimidine derivatives.

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