PHARMACOLOGY

Corrective Effect of the Bronchodilatatory Drugs Salbutamol and Troventol on the Regulatory Function of Lymphocytes from Atopic Bronchial Asthma Patients *in Vitro*

G. V. Poryadin, Zh. M. Salmasi, E. A. Busarova, and N. E. Zhuravleva

UDC 616.248-085.234-036.8-07:616.155.32-008.1

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.117, № 2, pp. 146-149, February, 1994
Original article submitted July 28, 1993.

It is shown that salbutamol and troventol possess bronchodilatatory and immunocorrective effects in bronchial asthma when the intracellular ratio of cyclic nucleotides in the organism is normalized.

Key Words: *salbutamol; troventol; atopic bronchial asthma*

The functional activity of human cells is largely determined by the balance of two cytonucleotide systems, cAMP/cGMP. In view of this, the physiological effects mediated by these cyclic nucleotides can be expected to change when their ratio is disturbed. In fact, the clinical use of selective β_2-adrenomimetics and M-cholinoblockers, acting via the system of cyclic nucleotides, has shown their high efficiency in mitigating bronchial asthma attacks. However, the effect of these drugs on changes of the immune status characteristic for atopic asthma is little known. There are a few reports describing the effect of β-adrenomimetics and cholinoblockers on individual parameters of the immune system in allergy, but unfortunately these studies have not led to a consensus on the immunotropic activity of these bronchodilatatory drugs.

The aim of the present investigation was to study the effect of new Russian-manufactured antiasthmatic drugs, namely the β_2-adrenomimetic salbutamol hemisuccinate and the M-cholinoblocker troventol, on regulatory lymphocytes in patients with bronchial asthma.

Russian State Medical University, Moscow. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Lymphocytes from the peripheral blood of 13 healthy persons aged 17 to 52 years and of 34 patients with the atopic form of bronchial asthma (ABA) aged 20 to 58 years were studied. Thirteen patients were in the stage of exacerbation and 21 patients were in remission. Diagnosis of house dust allergy was confirmed in all patients by allergologic examination (anamnesis, skin tests, provocational inhalation tests, presence of specific IgE-antibodies in the blood).

Isolation of lymphocytes was performed on a one-step Ficoll-Verographin density gradient ($\rho = 1.077$). The following monoclonal antibodies (MA) of the LT series (Institute of Immunology, Russian Ministry of Health) were used in the

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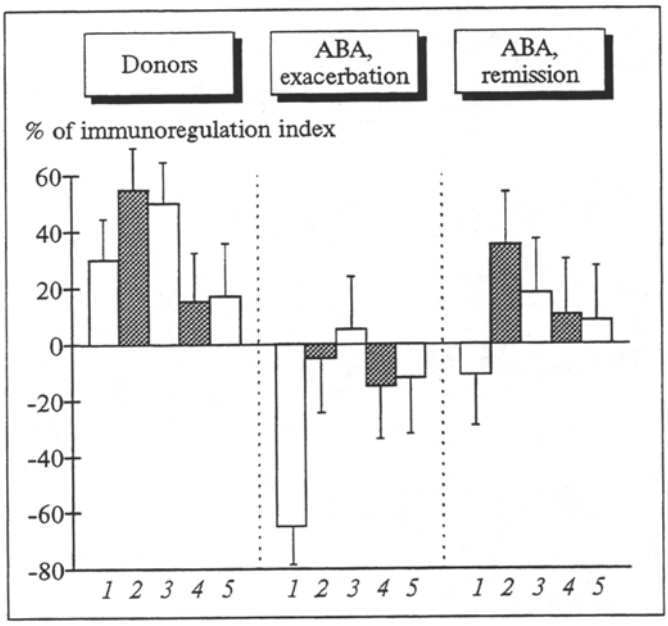

Fig. 1. Effect of salbutamol hemisuccinate (2 μg/ml) and troventol (0.1 μg/ml) on immunoregulatory lymphocytes from ABA patients: *1*) ConA; *2*) ConA+salbutamol; *3*) ConA +troventol; *4*) salbutamol; *5*) troventol.

study: LT1 against CD5 antigen, LT4 against CD4 antigen, and LT8 against CD8 antigen. The detection of fixed MA was performed using FITC-labeled rabbit antiserum against mouse immunoglobulins. Lymphocytes carrying surface immunoglobulins were detected using FITC-labeled rabbit antiserum against human immunoglobulins (Gamaleya Institute of Epidemiology and Microbiology). The microversion of the reaction was performed on glass.

For a study of the effect of the drugs on the lymphocyte populations and subpopulations monoclonal cells were incubated with the test drugs for 16 h in medium 199 at 37°C. Salbutamol was used in a concentration of 0.4 and 2 μg/ml and troventol at 0.02 and 0.1 μg/ml, which corresponded to the concentrations of these drugs in the blood when medium and maximal therapeutic doses are used in ABA patients.

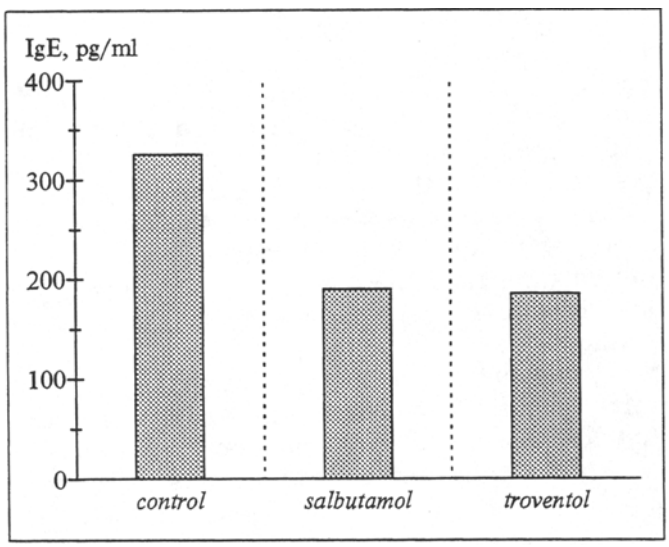

Fig. 2. Effect of salbutamol hemisuccinate (2 μg/ml) and troventol (0.1 μg/ml) on IgE production *in vitro* by lymphocytes from ABA patients.

The immunoregulatory function of T-lymphocytes was studied using the model developed by Shou et al. [3]. Concanavalin A (ConA) at 60 μg/ml was used as inductor. Salbutamol at 2 μg/ml and troventol at 0.1 μg/ml were added to cells before the beginning of culturing. The immunoregulation index was calculated according to the formula: IRI=1-T/C where T is the ^{3}H-thymidine incorporation in the test (cpm), and C is the ^{3}H-thymidine incorporation in the control. IRI<0% was specified as an index of suppression and IRI>0% as an index of activation.

Determination of spontaneous IgE synthesis was performed by culturing lymphocytes in penicillin flasks at 2 million/ml density in medium 199 with the addition of 10% fetal calf serum, 2 mM glutamine, 2 mM asparagine, and 10 mM HEPES for 7 days at 37°C. The addition of the test drugs to the cell culture was performed in the same manner as in the preceding experimental series. The content of total IgE in the supernatant was determined using a high-sensitivity immunoenzymatic test system developed at the Institute of Biotechnology under the guidance of R. G. Vasilov [5]. The level of *de novo* IgE synthesis was determined as the difference between the IgE content in cells and supernatants after the termination of culturing and the IgE content in cells before culturing. For determination of the IgE content in cells lymphocytes were destroyed by a multiple freeze-thawing procedure.

RESULTS

Salbutamol and troventol added to lymphocytes of healthy persons in the concentrations studied did not have any reliable effect on the content of lymphocyte populations and subpopulations (Table 1).

It is known that a drop of the $CD8^+$ lymphocyte content and a rise of the $CD4^+/CD8^+$ cell ratio are typical for patients with atopic diseases [9,10]. Salbutamol at 0.4 μg/ml added to lymphocytes from ABA patients in the stage of exacerbation produced a reliable rise of the $CD8^+$ lymphocyte content and a drop of the $CD4^+/CD8^+$ lymphocyte ratio. The number of $CD4^+$, $CD5^+$, and B lymphocytes did not markedly change (Table 1). Salbutamol in the same concentration did not cause

any appreciable changes in the content of lymphocyte populations and subpopulations in ABA patients in remission.

Incubation of lymphocytes from ABA patients in exacerbation with salbutamol at 2 μg/ml and 0.4 μg/ml was accompanied by changes having the same direction, but with the first concentration they were more pronounced. Thus, the number of $CD8^+$ lymphocytes increased from 15.93±0.23 to 23.21± ±0.31%, i.e., it practically reached the level of this parameter in healthy persons; the ratio $CD4^+/CD8^+$ lymphocytes dropped to the level of this parameter in donors (Table 1). In this concentrations salbutamol caused a significant rise of the $CD8^+$ lymphocyte content in patients in remission as well.

Troventol at 0.02 μg/ml did not produce reliable changes in the subpopulation composition of lymphocytes in ABA patients (Table 1), while at 0.1 μg/ml it caused a significant rise of the CD8+ lymphocyte number and a drop of the $CD4^+/CD8^+$ lymphocyte ratio both in patients in remission and in those in the exacerbation stage.

Therefore, salbutamol and troventol have a dose-dependent corrective effect on the subpopulation composition of lymphocytes from ABA patients, manifested in a reliable rise of the number of T lymphocytes carrying CD8 antigen on their membranes and in the normalization of the $CD4^+/CD8^+$ lymphocyte ratios. These changes are more pronounced in exacerbation of the disease.

The generation of T suppressors is impaired by superoptimal ConA doses in patients with bronchial asthma, as was shown previously in our studies dealing with the functional activity of immunoregulatory cells [3]. In the majority of patients ConA-stimulated immunoregulatory cells did not depress the proliferation of test lymphocytes, but even enhanced it (Fig. 1). The addition of salbutamol and troventol to a cell culture from ABA patients caused a reliable rise of T suppressor activity (Fig. 1). The index of ConA-induced immunoregulatory activity in asthma patients in exacerbation rises under the influence of salbutamol from -63.98±15.87 to -5.44±14.19% and under the influence of troventol to -5.84±9.76%. The activatory function of ConA-induced lymphocytes gives way to suppression under the effect of both drugs in patients in remission. The IRI rises from -

TABLE 1. Variation of Subpopulation Composition of Lymphocytes under the Effect of Salbutamol (SB) and Troventol (TV).

Antigen marker	Drug and its concentration, μg/ml	Group under study		
		healthy donors (n=13)	ABA patients in remission (n=12)	ABA patients in exacerbation (n=13)
$CD5^+$	control	56.17±1.06	56.82±0.84	56.18±0.68
	SB, 0.4	55.61±0.92	56.98±0.79	56.13±0.64
	SB, 2.0	55.98±0.87	58.12±0.78	57.02±0.60
	TV, 0.02	56.30±0.95	57.61±0.78	56.33±0.68
	TV, 0.1	56.60±0.78	56.71±0.85	56.70±0.71
$CD4^+$	control	43.71±1.08	44.63±0.98	44.41±0.54
	SB, 0.4	44.73±0.94	44.73±0.94	44.85±0.53
	SB, 2.0	44.34±0.94	46.88±0.70	45.61±0.59
	TV, 0.02	46.81±0.93	46.03±0.84	44.90±0.54
	TV, 0.1	46.40±0.91	46.04±0.76	45.42±0.61
$CD8^+$	control	24.76±0.54	17.38±0.47	15.93±0.23
	SB, 0.4	25.42±1.18	19.18±0.37	20.24±0.23**
	SB, 2.0	24.66±0.53	20.53±0.26*	23.20±0.31**
	TV, 0.02	24.98±0.46	17.81±0.45	17.87±0.21
	TV, 0.1	25.09±0.53	20.56±0.39*	20.34±0.24**
$CD4^+/CD8^+$	control	1.78±0.06	2.59±0.11	2.78±0.04
	SB, 0.4	1.81±0.04	2.33±0.08	2.21±0.02**
	SB, 2.0	1.81±0.05	2.29±0.04	1.97±0.03**
	TV, 0.02	1.89±0.06	2.60±0.09	2.50±0.03
	TV, 0.1	1.86±0.05	2.24±0.05*	2.22±0.04**
IgE^+	control	17.71±0.40	17.94±0.29	17.05±0.34
	SB, 0.4	18.15±0.44	17.95±0.30	17.25±0.34
	SB, 2.0	18.24±0.34	17.95±0.32	18.45±0.27
	TV, 0.02	18.18±0.36	18.06±0.30	17.78±0.28
	TV, 0.1	19.22±0.37	19.03±0.36	18.12±0.31

Note. Asterisks signify the reliability of differences: * — $p<0.05$, ** — $p<0.01$.

12.01±6.74 to 34.17±4.51% for salbutamol and to 17.02±10.44% for troventol. The suppressive function of ConA-induced lymphocytes is boosted by salbutamol and troventol in healthy persons as well (Fig. 1). At the same time, neither salbutamol nor troventol in the concentrations studied caused reliable changes of spontaneous immunoregulatory cells being incubated without ConA (Fig. 1).

Since salbutamol and troventol caused changes of the same direction in different experimental models, we assumed that normalization of the regulatory component of the immune system should also be reflected in the synthesis of the main atopic allergic immunoglobulin IgE. We found that salbutamol and troventol caused a significant drop of IgE production by patients' lymphocytes from 325.62±59.62 to 194.14±33.32 pg/ml for the former and to 188.25±42.52 pg/ml for the latter (Fig. 2).

Since the immunocorrective effect of salbutamol and troventol is similarly manifested in different immunologic models, the question arises as to what mechanisms mediate the effect of these drugs on the immune system. The comparison of our present findings with the data on the effect of other pharmacological agents on regulatory T lymphocytes from asthma patients [4,6,11,12] leads to the conclusion that the effect of salbutamol and troventol is related to their influence on the metabolism of cyclic nucleotides in the cell. The initial factor underlying the damage of T suppressor function in atopic asthma patients is probably the imbalance of the ratio between these secondary messengers [7]. This assumption explains the results obtained, because the system of cyclic nucleotides mediates not only cell proliferation and differentiation, but also the transmission of positive or negative signals to cell receptors, thereby determining the expression or reexpression of antigens on the lymphocyte membrane. The direct cause of the development of the cAMP/cGMP imbalance in lymphocytes from ABA patients may be both the damage of transmitter reception, primarily altered β-receptor function [13], and the effect of biologically active substances released by target cells in the allergic reaction [1].

Thus, by normalizing the intracellular ratio of cyclic nucleotides in the organism in bronchial asthma, salbutamol and troventol manifest not only a bronchodilatatory, but also an immunocorrective effect.

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