EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Correlation between the Results of Glucocorticoid Therapy and *in Vitro* Effect of Glucocorticoids on Monocytes in Asthma

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The effects of glucocorticoids on monocyte morphology and function *in vitro* and the results of high-dose budesonide therapy in patients with non-severe bronchial asthma were analyzed. Before therapy with inhalation glucocorticosteroid (budesonide) characteristics of blood monocytes and the effects of different concentrations of prednisolone on these cells were studied *in vitro* by luminol-dependent chemiluminescence and computer-assisted phase-interference microscopy. High sensitivity of patients to budesonide was associated with pronounced *in vitro* inhibitory effect of prednisolone on monocyte activity, which was not observed in cases with delayed effects of therapy. Pronounced inhibitory effects of glucocorticoids on monocytes *in vitro* were observed in patients both resistant and highly sensitive to glucocorticoid therapy. Hence, the resistance of patients with non-severe asthma to high-dose budesonide therapy is not related to the weakening of the inhibitory effect of glucocorticoids on monocyte activity.

Key Words: *asthma; monocytes; chemiluminescence; computer-assisted phase-interference microscopy*

Inhalation glucocorticosteroids (GC) form the basis of antiinflammatory therapy of asthma. The development and clinical testing of noninvasive methods for evaluation and monitoring of the inflammatory process suitable for validation of the use of GC in these are now in progress. Among these methods are NO and H_2O_2 assay in the exhaled air, eosinophil count in induced sputum, measurements of blood eosinophil cationic protein, *etc.* [7].

Multicellular inflammatory process in asthma involves resident cells of the airways and recruited secondary effector cells (eosinophils, basophils, lymphocytes, and monocytes) [2,3]. Mononuclear phagocytes (monocytes, monocyte-derived macrophages, and dendritic cells) play an important role in the pathogenesis of asthma [6]. Monocytes and macrophages in asthma express high affinity IgG receptors (Fc_eR1) and can be activated by multivalent allergens [4,8]. Spontaneous production of granulocyte-macrophage colony-stimulating factor and increased level of reactive oxygen species attest to activation of monocytes in asthma [9,13].

GC in asthmatics produce an indirect effect on eosinophils by inhibiting production of cytokines by monocytes, epithelial cells, and fibroblasts [5,10,12]. M. Schmidt *et al.* [11] showed that antiinflammatory

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effect of GC is at least partially related to induction of monocyte apoptosis.

We studied the morphology and functions of monocytes, their reactions to GC *in vitro*, and the correlation between cell sensitivity to GC and the results of glucocorticosteroid therapy in patients with non-severe asthma.

MATERIALS AND METHODS

The study was carried out in 42 patients (22 women and 20 men aged 16-69 years) with persistent mild (n=4) and moderate (n=38) asthma. None of them was previously treated with GC. The therapy with long-acting theophylline in standard doses was insufficient: clinical manifestations of asthma did not disappear and symptomatic therapy with short-acting β_2-agonists was needed. During the study the patients stayed in the hospital under conditions practically excluding the contact with allergens and received standard inhalation GC (budesonide) therapy (using Benacort powder inhalator) in a daily dose of 1000 μg. The efficiency of GC inhalation therapy were evaluated by the severity of objective manifestations of the disease (coughing, whistling, and dyspnea scored from 0 to 4 points), physical symptoms, need in symptomatic therapy with short-acting β_2-agonists, and data of functional monitoring carried out with a Minitest electron spirometer (forced expiratory volume in the first second and forced vital capacity).

The morphology and function of peripheral blood monocytes were evaluated by luminol-dependent chemiluminescence (CL) and computer-assisted phase-interference microscopy [1] before budesonide treatment. The blood was collected from the ulnar vein after overnight fasting and mononuclears were isolated from the blood in Ficoll-verografin by A. Boyum's method and diluted to a concentration of 10^6/ml with Hanks' solution. These cells and the cells from the same suspension preincubated with prednisolone (10^{-7} and 10^{-4} mmol/liter, 60 min, 20°C) were used for CL analysis. The cell suspension (850 μl) and 0.1 M luminol (100 μl) were put in a thermostated cuvette of CLM-3 chemiluminometer and the maximum spontaneous chemiluminescence (CL_{SP}) was recorded. After 5 min 50 μl zymosan suspension opsonized with human serum was added and the maximum activated chemiluminescence (CL_{ACT}) was recorded (Fig. 1). The degree of CL inhibition with prednisolone was evaluated by calculating CL depression indexes using the following formulae:

CL_{SP} depression index=$[CL_{SP(IN)}-CL_{SP}(10^{-4})]/CL_{SP(IN)}$
and
CL_{ACT} depression index=$[CL_{ACT(IN)}-CL_{ACT}(10^{-4})]/CL_{ACT(IN)}$.

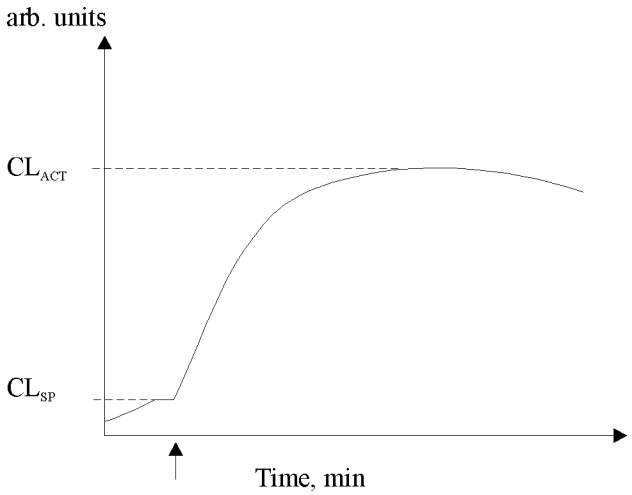

Fig. 1. Chemiluminescence curve and parameters of phagocytizing cell. The arrow shows time of addition of zymosan into the medium.

TABLE 1. CL of Peripheral Blood Mononuclear in Groups of Patients with Asthma before and after Incubation with Prednisolone in Different Concentrations

Conditions of CL analysis in groups		CL parameters	
		CL_{SP}	CL_{ACT}
1	initial	0.06±0.03	0.73±0.2
	prednisolone (10^{-7} mmol/liter)	0.05±0.03	0.53±0.18
	prednisolone (10^{-4} mmol/liter)	0.03±0.02	0.41±0.18
2	initial	0.05±0.02	0.76±0.15
	prednisolone (10^{-7} mmol/liter)	0.03±0.01	0.61±0.14
	prednisolone (10^{-4} mmol/liter)	0.06±0.03	0.60±0.12
3	initial	0.060±0.017	0.59±0.15
	prednisolone (10^{-7} mmol/liter)	0.020±0.006*	0.32±0.14
	prednisolone (10^{-4} mmol/liter)	0.020±0.007*	0.20±0.06*

Note. *p<0.05 compared to initial values.

TABLE 2. CL Depression Indexes in Different Groups of Patients with Asthma

Group	CL_{SP} depression index	CL_{ACT} depression index
1	0.66±0.08*	0.59±0.11***
2	0.12±0.10	0.13±0.18
3	0.57±0.16***	0.72±0.10**

Note. *p<0.001, **p<0.01, ***p<0.05 compared to group 2.

In order to discriminate between the initial CL and CL after incubation with prednisolone in concentrations of 10^{-7} and 10^{-4} mmol/liter, the former is shown as CL_{in} and the latter with prednisolone concentrations (10^{-7} or 10^{-4}, respectively).

Control group consisted of 8 donors (nonsmokers). The results were recorded and evaluated using Chart v3.4.3 software and MacLab-Macintosh computer system.

Life-time computer-aided phase-interference microscopy of monocytes was carried out in 16 asthmatics and 10 donors. The study was carried out using Russian computer-aided laser phase-interference microscope Cytoscan (modified Linnik interferometer with reference wave phase modulation). He-Ne laser (λ=633 nm) served as the source of light. The cells were isolated from the peripheral blood and incubated with prednisolone similarly as for CL analysis. In each sample 50-100 cells were analyzed.

Complex algorithm of phasometry allowed automated morphometry of cells (diameter, perimeter, height, area, and volume), visualization of phase-interference cell image (Fig. 2; topogram, three-dimensional image, profile, histogram of phase heights distribution), statistical data processing, and recording of the results in protocols.

TABLE 3. Morphometric Parameters of Monocytes before and after Incubation with Prednisolone

Parameters	Groups		
	1 (n=5)	2 (n=6)	3 (n=5)
D_{IN}, μ	6.8±0.2	7.0±0.2	6.7±0.3
$D(10^{-7})$, μ	5.9±0.2*	7.2±0.2	6.7±0.3
$D(10^{-4})$, μ	6.48±0.19	7.08±0.23	6.68±0.23
P_{IN}, μ	20.3±0.5	21.1±0.5	19.9±0.9
$P(10^{-7})$, μ	18.3±0.5*	21.5±0.5	20.2±0.8
$P(10^{-4})$, μ	19.40±0.58	21.2±0.8	20.1±0.7
H_{IN}, μ	2.1±0.1	2.3±0.1	2.2±0.1
$H(10^{-7})$, μ	1.9±0.1	2.4±0.1	2.2±0.1
$H(10^{-4})$, μ	1.96±0.09	2.30±0.06	2.06±0.10
S_{IN}, μ^2	30.4±1.4	32.4±1.6	28.8±2.2
$S(10^{-7})$, μ^2	24.1±1.4*	33.9±1.5	29.5±2.1
$S(10^{-4})$, μ^2	28.08±1.70	32.9±2.4	29.3±1.7
V_{IN}, μ^3	32.8±3.0	43.5±3.8	34.2±3.5
$V(10^{-7})$, μ^3	25.80±2.16	45.9±3.5	34.9±3.7
$V(10^{-4})$, μ^3	29.36±3.30	40.2±2.9	32.5±2.3

Note. *p<0.05 compared to initial values. D: diameter; P: perimeter; H: height; S: area; V: volume.

RESULTS

The results of therapy were as follows: complete clinical and functional effect of GC treatment within 9 days of budesonide therapy in 11 patients (group 1); delayed effect (12-36 days) in 23 patients (group 2); incomplete clinical and functional effect over 40-day stay in hospital and subsequent 3 months of outpatient treatment (n=8, group 3).

The initial parameters of baseline CL in asthmatics and donors differed significantly ($CL_{SP(IN)}$ 0.057±0.011 and 0.033±0.005, respectively, p<0.05), which indicates activation of circulating monocytes in asthma, while $CL_{ACT(IN)}$ was virtually the same (0.71±0.14 and 0.67±0.05, respectively).

The initial parameters of CL were the same in patients with different efficiency of GC therapy, while after incubation of mononuclears with prednisolone in different concentrations some differences between groups were revealed (Table 1). In group 1 CL_{SP} and CL_{ACT} progressively decreased with increasing prednisolone concentration. In group 2 no appreciable changes in CL parameters were observed. In group 3 CL_{SP} and CL_{ACT} of incubated cells markedly decreased. When comparing CL depression indexes we found that the decrease of CL parameters in group 2 was more pronounced than in groups 1 and 3 (Table 2), which confirmed slight inhibitory effect of prednisolone on monocyte activity *in vitro* in group 2 patients.

Comparison of morphometric parameters of peripheral blood monocytes in asthmatics and donors showed significant differences in cell diameter (6.83±0.12 and 7.28±0.12, respectively, p<0.05) and area (30.64±1.01 and 34.71±0.82, respectively, p<0.01). Incubation of monocytes from group 1 patients with 10^{-7} mmol/liter prednisolone led to a significant decrease in cell diameter, perimeter, and area. In other groups no significant changes in morphometric characteristics of monocytes were detected (Table 3).

High level of $CL_{SP(INIT)}$ in asthmatics agrees with published data on spontaneous hyperproduction of reactive oxygen species by blood monocytes in this pathology. Some morphometric characteristics of monocytes are also different in asthmatics and donors.

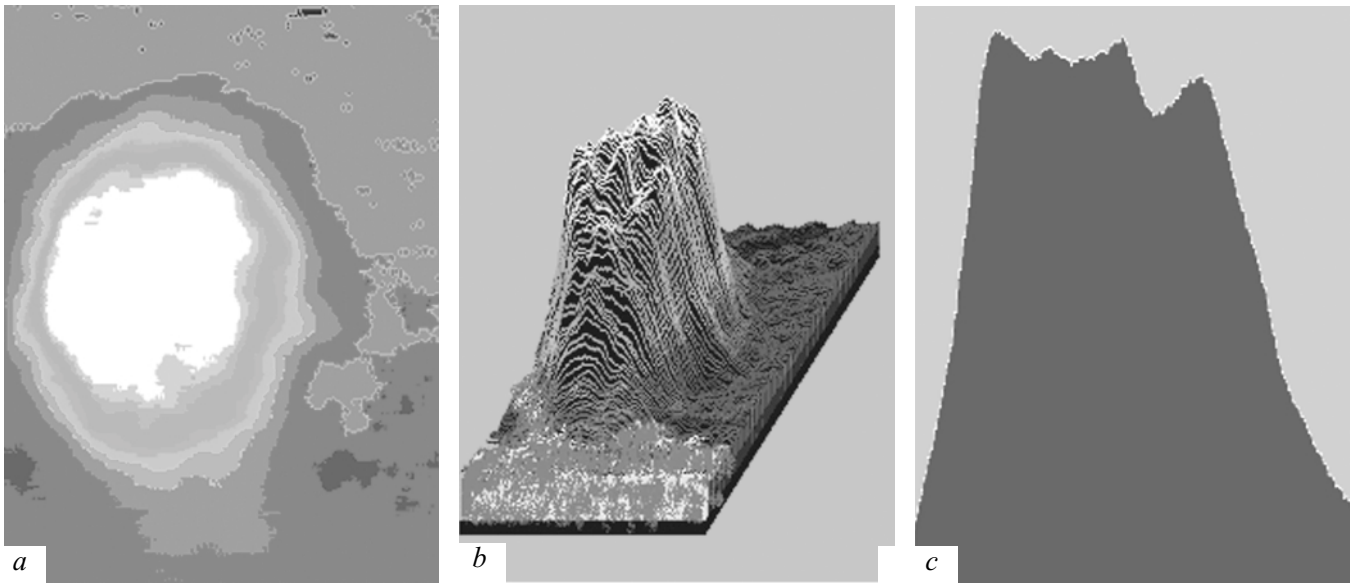

Fig. 2. Life-time phase-interference image of a monocyte. *a*) topogram; *b*) three-dimensional image; *c*) cell section.

High sensitivity to budesonide therapy was associated with pronounced depression of monocyte CL and significant changes in some of their morphometric parameters after *in vitro* incubation with prednisolone. In group 2 the inhibitory effect of GC on monocyte activity *in vitro* was lower than in group 1. In group 3 clinical symptoms did not disappear during budesonide therapy, though monocytes from these patients were highly sensitive to GC *in vitro*. We believe that the course of the disease in these patients can be determined by some non-inflammatory mechanisms.

Hence, signs of activation of circulating monocytes (hyperproduction of reactive oxygen forms) were observed in patients with non-severe asthma. A significant correlation between the depression of monocyte activity by GC *in vitro* and the time of attaining clinical and functional remission during high-dose budesonide therapy was detected in steroid-sensitive patients with non-severe asthma. This opens good prospects for individual planning of treatment based on the results of preliminary laboratory analysis. The mechanisms underlying the resistance to inhalation GC in non-severe asthma were not related to weakening of the inhibitory effect of GC on monocyte activity and deserve further investigation.

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