HUMAN COMPLEMENT SYSTEM STATE AFTER WOBENZYME INTAKE

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Wobenzyme is a preparation, which contains proteinases of animal (trypsin, chymotrypsin) and plant (papain, bromelain) origin. Healthy people and patients with colon pathology were given 5–10 wobenzyme pellets. Some indices of the complement hemolytical activity (the velocity of complement-dependent hemolysis via the classical pathway, the velocity of complement-dependent hemolysis via the alternative pathway and the index of reactive lysis) were determined in their sera samples before and 1–2 h after wobenzyme intake. In healthy people and in patients with the syndrome of irritated bowel no change of complement activity occurred after wobenzyme intake. In the patients with nonspecific ulcerative colitis the index of reactive lysis was rather high and appeared to diminish after wobenzyme intake. The other parameters of complement activity did not alter.

Wobenzyme is a pharmacological preparation, produced by Mugos-Pharma (Germany) for the systemic enzymatic therapy. Each pellet contains four proteinases: trypsin (24 mg), papain (60 mg), bromelain (45 mg) and chymotrypsin (1 mg). Besides, it contains rutin, pancreatin, pencreatic amylase and lipase. According to Veremeenko [1] proteinases of the preparation in some extend penetrate from intestine to blood stream where they complex to endogenous proteinase inhibitors including α_2-macroglobuling. The author found an elevation of plasma proteolytic activity towards protamin sulfate in 1–4 h after peroral wobenzyme administration to rabbits. The rise of plasma proteolytic activity can be explained by the appearance of non-bound proteinases when they prevail the endogenous proteinase inhibitor potential. The elevation of plasma proteolytic activity in its turn can cause the damage of some plasma proteins. The effectiveness of wobenzyme treatment is partially explained by destruction of plasma proteins such as immune complexes [2].

The complement system is a unity of several plasma proteins performing mainly an immune cytolysis. Some complement proteins can serve as the substrates for trypsin [3] or other low specific proteinases [4]. They are expected to be digested by wobenzyme proteinases entered to the bloodstream. The aim of the study was to reveal the possible change of complement functional activity after wobenzyme intake by healthy people or patients.

Methods

The groups of patients, suffering with colon diseases, and healthy people were given 5–10 pellets of wobenzyme followed by 200 ml of water. Blood samples were taken before and 1.5–2.0 h after preparation intake. The most the patients continued wobenzyme treatment for 3–4 weeks. After that, they were examined clinically. The indices of complement functional activity [5] such as the velocity of complement-dependent hemolysis via the classical pathway (CPC), the velocity of complement-dependent hemolysis via the alternative pathway (APC) and the index of the reactive lysis (characterizing the complement membrane attack complex damage of the cells bystander to the complement initiators) were determined in sera samples. In the course of the reactive lysis some cells or polymeric molecules initiate the complement cascade to destroy the innocent cells in some pathology—the self ones. The suspension of washed (3 times) rabbit erythrocytes in veronal buffer solution (pH 7.2) was used for complement initiation. The parameters of the complement hemolytical activity were determined in termostated (37°C) flask of the photometer monitoring the optical density change at 800 nm. For APC parameters determination the incubation mixture was supplied with EGTA (10 mM) to block CPC by calcium chelation. Non-parametric method of Willcockson-Mann-Witney was used for statistical data analysis.

Results and Discussion

The parameters of the complement functional activity were determined in three groups of people before and after wobenzyme intake. The patients with nonspecific ulcerative colitis (NUC) represented the first group. The disease is of autoimmune nature and of unknown etiology. Inflammation reaction and multiple colon ulcers manifest it. The second group of patients had the syndrome of irritated bowel (SIB). In this pathology, the colon dysfunction is not connected with any morphological disorders. The third group was composed with practically healthy persons. The results are represented in the table.

According to our data wobenzyme intake by healthy people and the patients, suffering the syndrome of irritated bowel, did not influence the functional state of the complement system. Probably the preparation enzymes, if appeared in their active form in blood, are not enough

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Table

The indices of human complement hemolytical activity before and after wobenzyme intake
(NUC—nonpecific ulcerative colitis, SIB—syndrom of irritated bowel,
CPC—classical pathway of complement, APC—alternative pathway of complement)

Groups of people	n	The velocity of complement-dependent hemolysis via CPC (mln cells per min) M ± m		The velocity of complement-dependent hemolysis via APC (mln cells per min) M ± m		The index of the reactive lysis, M ± M	
		Before	After	Before	After	Before	After
NUC	25	54 ± 8	51 ± 6	8.1 ± 1.4	9.3 ± 2.2	3.3 ± 0.2	2.5 ± 0.2*
SIB	17	40 ± 11	40 ± 12	10.2 ± 2.6	9.5 ± 2.8	2.0 ± 0.4	2.1 ± 0.1
Healthy	6	45 ± 6	42 ± 6	9.8 ± 4.2	7.4 ± 1.6	1.8 ± 1.4	2.5 ± 0.1

* $p < 0.01$.

to destroy essential quantity of complement components to achieve the functional response. In the patients, suffering with nonspecific ulcerative colitis, a statistically valid change after wobenzyme intake was found for only one parameter. This parameter was the index of reactive lysis, which substantially lowed in the most of patients. The results of clinical examination showed that wobenzyme treatment was especially effective in those patients who had demonstrated the most pronounced lowering of the index after wobenzyme intake.

There are many literary data about complement involvement in the pathogenesis of nonspecific ulcerative colitis. In damaged colon epithelium and submucosa the fragments of activated complement (C3b, C3c, iC3b/C3dg) and complement membrane complexes were revealed with immunochemical technique [6, 7]. Their association with immunoglobulin G deposits [7] show that specially modified colon cells in this pathology can initiate the classical pathway of complement and be damaged by the complement. During complement activation, its components undergo proteolysis to be exhausted. But in blood plasma the quantity of complement components is not diminished but even elevated [8]. It is due to acceleration of complement protein synthesis including the increase of that synthesis in an inflamed bowel. An elevation of plasma C3 level in nonspecific ulcerative colitis was observed to correlate with the disease activity and the concentration of C-reactive protein [9]. The authors also marked the tendency to an increase of the complement hemolytical activity of the patients' sera. Our data also show an acceleration of complement-dependent hemolysis via CPC in most patients that did not fall after wobenzyme intake.

Acceleration of both complement degradation and synthesis, characteristic for nonspecific ulcerative colitis, must induce a reactive lysis. Blood plasma and membrane surfaces contain special proteins with protect human cells from the self-complement attacks [10, 11]. It was probably due to a compensatory effect that those proteins appeared to be elevated in nonspecific ulcerative colitis [12]. Nevertheless, the membrane attack complexes depositions in the sites of inflammatory colon damage evidence an insufficiency of the anticomplement system. It is also evidenced by the success of nonspecific ulcerative colitis treatment with a complement system inhibitor [14]. The lowering of the index of reactive lysis after wobenzyme intake, found in our study, must restrict complement-dependent cell damage and provide the clinical effectiveness of the preparation in nonspecific ulcerative colitis. Therefore, our data show that the clinical effectiveness of wabenzyme in nonspecofoc ulcerative colitis may be partially due to the restriction of complement reactive lysis.

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