USE OF THE IMMUNO-ENHANCER GEPON FOR THE TREATMENT OF ULCERATIVE COLITIS

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The efficacy of the immuno-enhancer Gepon was studied for treatment of patients having distal forms of Ulcerative Colitis, such as ulcerative proctitis and proctosigmoiditis. 36 patients with Ulcerative Colitis, resistant to the conventional anti-inflammatory therapy, have been participated in this study.
Use of Gepon in the drug forms of rectal suppository or micro-enema led to a significant improvement in majority of patients. Symptoms of Ulcerative colitis have been declined and the remission achieved in 65% patients with ulcerative proctitis and 70% patients with ulcerative proctosigmoiditis. Besides, use of Gepon led to a normalisation of patients' immune status parameters. Based on the results of this study we recommend a use of Gepon for treatment of Ulcerative Colitis patients.

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Ulcerative colitis (UC) is a serious medical and social problem with increasing morbidity. UC and its serious complications lead to the early invalidization of young persons of working age. The prevalence of relapsing UC varies between 28 to 117 per 100,000 of the population [1, 13]. The distal forms of UC, which include proctitis and proctosigmoiditis, comprise 40-60% of patients [2, 6, 7, 10]. In spite of intensive study, many questions regarding UC remain unanswered. Although specific success in the clinical diagnosis of UC has been achieved; therapy of this disease is often unsuccessful [11, 12]. The search for the solution to the problem has been hindered, because the etiology and pathogenesis of UC has not been established.

Contemporary treatment of UC is reduced to simply the suppression of inflammation. The basic therapies for UC are corticosteroids and preparations of 5-amino salicylic acid (5-ASA). However, in a number of cases the use of these preparations is not effective [3, 14-17]. The drug resistance results in chronically active form of the UC, such patients loose their ability to work, and the disease is transformed into more serious forms of UC [4, 14, 15]. With distal forms of UC, resistance to treatment is frequently encountered, and remains one of the urgent problems of gastro-enterology and coloproctology

Immunological mechanisms are believed to have a key role in pathogenesis of UC [1, 3, 7, 11]. Therefore, many researchers have turned their attention to pharmaceuticals with immuno-correcting properties. In connection with this direction of research, we studied the safety and efficacy of the immuno-modulator Gepon in patients suffering chronic distal UC, its constantly active form.

The pharmaceutical Gepon was selected because of its anti-inflammatory, anti-infective and tissue regenerative properties. According to the data in the literature, Gepon has been successfully used for the treatment of a number of the diseases of the gastrointestinal tract, such as irritable bowel syndrome, stomach and duodenal ulcers, and also acute intestinal Infections both of viral and bacterial etiology [5, 8, 9].

MATERIALS AND METHODS

The study included 36 patients with UC (19 men, 17 women, age from 22 to 69 years, an average of 45.5 years). The patients have been receiving in-hospital treatment during the period 2003-2004 at the State Scientific Centre for Coloproctology, Ministry of Health, Russian Federation.

In all patients the distal form of UC was confirmed on the basis of endoscopy, colonoscopy with the biopsy and histological investigations. Proctitis was found in 26 patients, and proctosigmoiditis in 10 patients. The degree of gravity of UC was determined by the Schroeder Index, (Mayo Clinic, University of California, DAI), taking into account the following criteria:

1) a frequency of defecation in the twenty-four hours quantified as follows:
      0 marks: 1-2 defecation a day;
      1 mark: 3-4 defecation a day;
      2 marks: 5-6 defecation a day;
      3 marks: 7 and more defecation a day.

2) rectal hemorrhage:
      0 marks: none
      1 mark: a little blood in less than half of defecations;
      2 marks: blood in the majority of defecations;
      3 marks: only blood.

3) rectoromanoscopy examination data:
      0 marks: remission;
      1 mark: hyperemia, the decrease of vascular network, weak looseness;
      2 marks: hyperemia, absence vascular network, looseness, erosion;
      3 marks: spontaneous hemorrhage, ulcers.

4) complete physical estimation (according to Truelove Witts and M.X. Levitano):
      0 marks: standard;
      1 mark: light aggravation;
      2 marks: aggravation of average gravity;
      3 marks: heavy aggravation.

A gravity of UC was represented as the UC activity index which was calculated as a sum of the above marks:
      0-2 marks: remission;
      3-5 marks: mild case;
      6-8 marks: moderately heavy form;
      9-12 marks: heavy form.

In accordance with the described criteria of the UC gravity in this investigation, mild cases (3-5 marks) were established in 20 patients (56%), and moderately heavy forms of UC (6-8 marks) were established in 16 patients (44%).

As mentioned above, 10 patients had ulcerative proctosigmoiditis form of UC. In 7 of the 10 patients (70%), the frequent (more than 3-4 times in a 24 hour period) stools mixed with blood, and false urges for defecation with the delivery of blood-containing mucus or blood (up to 3 to 5 times in a 24 hour period) were predominant complaints in the clinical picture of the disease. The rectoromanoscopy examination showed a hyperemia of the friable rectal mucus membrane, the vascular network of which was absent, and erosions were observed. The status of patients corresponded to the moderately heavy degree of gravity and the UC activity index was 6-8 marks.

In 3 of 10 patients (30%), the clinical picture showed a tendency to constipation (stools once in 2-3 days with lines or explicit admixture of blood), and periodic false urges with the isolation of blood-containing mucus. According to the data of the rectoromanoscopy, there was hyperemia of the friable mucus membrane, decrease of vascular network, and the mucus membrane of the rectum was easily wounded on contact with the rectoscope. The status of patients corresponded to a mild degree of gravity, 3-5 marks on the UC activity index.

Ulcerative proctitis was confirmed in 26 patients. In 17 of 26 patients (65.4%) with ulcerative proctitis, the complaints of bloody mixture in their stools predominated rather than more frequent stools (frequency of defecation of 1-2 times per day), or of constipation for 2-3 days and periodic false urges for the defecation with the outcome of blood-containing mucus or visible blood. The rectoromanoscopy examination showed hyperemia of the rectal mucosa, and absence of the vascular network in some loci, as well as looseness and contact vulnerability of mucosa. The status of these patients corresponded to a mild degree of gravity, the UC activity index was 3-5 marks. In 9 of 26 patients (34,6%) the clinical picture was predominantly false urges 3-4 times in a 24 hour period, stools was made more frequently 3-4 times in a 24 hour period, in small portions, liquid, with traces of blood, or an explicit admixture of blood. According to the rectoromanoscopy, hyperemia of the friable rectal mucosa, and absence of vascular networks, and plural small erosions have been recorded. On contact with the rectoscope mucosa was easily hurt. The status of patients corresponded to the moderate heavy form of UC, the index of activity was 6-8 marks.

A correspondence between the UC activity index and topological diagnosis has been found. The ulcerative proctosigmoiditis had higher activity indexes than the ulcerative proctitis (Fig. 1).

All 36 patients assigned to the Gepon treatment study, had failed to respond to standard anti-inflammatory treatment and their UC was resistant to the local applications of 5-ASA-preparations and corticosteroids. The preparation of Gepon was delivered either in the form of rectal suppositories or micro-enemas. Gepon was a sterile lyophilized preparation in vials (0.002 g) produced by OOO "Immapharma" (Moscow). For the micro-enemas the preparation was dissolved directly before use in 40 ml of sterile physiological solution (0.85% NaCl). The Gepon suppositories were prepared in the hospital pharmacy of the State Scientific Centre for Coloproctology

(Ministry of Health, Russian Federation), and the composition of one suppository was Gepon 0.001 g, cocoa butter 1.5 g.

The patients were divided into 3 groups to receive the different doses and routes of administration of Gepon.

Group-1 (n = 21) patients with the proctitis form of UC; Gepon was administered in the form of rectal suppositories; one suppository a day, for a total course of treatment with 4 suppositories.

Group-2 (n = 5) patients with the proctitis form UC; Gepon was administered in the form of rectal suppositories one suppository a day, during 7 days, a total course of treatment with 7 suppositories.

Group-3 (n = 10) patients with the proctosigmoiditis form of Ulcerous Colitis; Gepon was administered in the form of micro-enemas, 0.002 g daily during 7 days.

For the duration of Gepon treatment, basic treatment with anti-inflammatory 5-amino-salicylic acid preparations and corticosteroids were abolished. After the end of treatment with the Gepon preparations, all patients were transferred back onto anti-inflammatory 5-ASA-preparations and local applications of corticosteroids.

The indices of immune status were investigated before treatment and 7 days after the end of treatment. Immune status was studied using laser cytometry, chemoluminescence, and immunodiffusion. The phagocytic activity of blood neutrophiles was measured using phagocytosis of *E. coli* labelled with fluorescence and the subsequent evaluation of results using laser flow cytometry. Functional response of neutrophils to opsonised zymosan and PMA was studied by the chemoluminescence of cells.

RESULTS OF THE STUDY

In 24 (92%) of 26 sick patients, comprising Group-1 and Group-2, after the application of only the first suppository of Gepon, there was observed a positive clinical effect, with the disappearance of false urges and a decrease in the admixture of blood in the stools (Table 1 and Fig. 2). The positive result obtained, remained during the continuation of the treatment in 17 (65%) of the patients. Of these sick patients, 10 patients achieved clinical remission, and 7 showed total clinico-endoscopic remission of disease.

The other 7 (27%) patients responded to Gepon treatment with only transient improvement. They had clinical improvement after the first suppository, but relapsed back into the previous state during the continuation of treatment. Nevertheless, in 4 of 7 patients, it was possible to achieve a clinical remission from UC by continuing with local 5-ASA-preparation immediately after the end of treatment with Gepon suppositories (after the end of Gepon treatment, the patients were administered with suppositories of Salofalk 1 g a day over one month). The remaining group of 3 patients did not succeed in reaching the remission from disease, in spite of the continuation of a one-month course of treatment with Salofalk suppositories, and continuous therapy with prednisolone at a dose of 20 mg/day for another month. The chronic-continuous course of disease persisted in these patients.

In 2 (8%) of 26 sick patients who stopped existing therapy and received Gepon suppositories, their condition worsened, which was expressed in quickening of the stools and false urges from 2-4 times a day to 7-10 times a day, with an increase in the admixture of blood. An increase in the UC activity index on endoscopy from 3-6 to 4-8 marks was noted, and also an increase in the activity of inflammatory process according to the clinical and biochemical signs: falling hemoglobin and erythrocytes, an increase in the number of leukocytes, increase in erythrocyte sedimentation reaction, and the level of fibrinogen in peripheral blood. In these 2 cases, the treatment with Gepon was stopped and treatment with suppositories of prednisolone at the dose of 20 mg/day and suppositories of Salofalk at 1 g/day was resumed. A subsequent improvement of these two patients was observed, UC symptoms and activity indexes had declined.

The comparison of Gepon clinical effects in Group-1 and Group-2 did not reveal any significant differences between the groups (Table 1). From this, it is possible to conclude that the two courses of the treatment used - on 1 suppository of Gepon a day for either 4 days or 7 days - had identical clinical effectiveness.

In Group-3, clinico-endoscopic improvement was noted in 9 (90%) of 10 patients with ulcerative proctosigmoiditis, who received Gepon as micro-enemas (Table 2 and Fig. 3). In 2 of 9 patients (20%), the positive effect was short-term, and only observed after the first micro-enema, then the symptoms of disease appeared again. After the end of treatment with Gepon micro-enemas, hydrocortisone 125 mg/day and suppositories with prednisolone 20 mg/day, were administered for one month after which there was a remission of disease. In 7 of 9 patients (70%) achieved a positive clinical effect from the Gepon micro-enemas which proved to be stable: clinical and endoscopic manifestations of the UC were absent both at 7 days and 1 month after the completion of treatment. The UC activity index dropped to '0' mark in 6 of the patients and down to '1' mark in the remaining patient. Only 1 of the 10 sick patients (10%), treated with Gepon micro-enemas did not gain any positive benefit.

Changes in immune status under influence of treatment with Gepon

The initial immune status of patients with UC, before the treatment with Gepon, was characterized by significant changes in the populations of NK cells and of cytolytic CD8$^+$ T-cells.

NK cells. In the UC patients involved in this study the total counts of NK cells proved to be within the limits of the allowed values (15 $\pm$ 4%, 348 $\pm$ 219 cells per 1 µl). In spite of this, it was obvious there were series of changes in this cellular population, related to the different isotypes of NK cells. In spite of the massive activation of NK cells, their cytolytic function was clearly weakened. The counts of cytolytic NK cells having intracellular perforin was below standard in 47% of patients with UC. The NK cells response had apparently reached a phase of decompensation, as is always the case during the course of very prolonged but unsuccessful defensive reactions by the cells of the immune system.

CD8$^+$ T cells. The population of CD8$^+$ T cells in patients with UC was characterized by hyperplasia and activation, and differentiation into the effector cells, in particular into cytolytic T cells. The average content of CD8$^+$ T cells in the peripheral blood of the inspected patients had reached 28% or 648 cells in 1 µl. In 35% of patients the counts of CD8$^+$ T cells exceeded the upper boundary of standard range (K = 1,17) and the CD8$^+$ T cells population was clearly activated. This was identified by the intensive expression of the activation markers on the surface CD8$^+$ T cells. In 59% of the subjects, there was an increased content of activated CD8$^+$ T cells, which expressed HLA-DR molecules. The intensive expression of the interleukin-2 receptor (CD25) proved activation of CD8$^+$ T cells in 47% of patients, and the simultaneous expression of surface CD45RO and CD45RA confirmed the activation CD8$^+$ T cells in 44% of the patients.

The activation of CD8$^+$ T cells in patients with UC was accompanied by the maturation of these cells into full-fledged effector T cells. The process of maturation was manifested by the shedding of CD28 molecule from the surface CD8$^+$ T cells in 50% of patients with UC and by the passage of "naive" CD8$^+$CD45RA$^+$ T cells into CD8$^+$CD45RO$^+$ T "memory" cells in 31% of subjects. Transformation into functionally mature effector T cells was also documented by the increased counts of perforin-positive cytolytic CD8$^+$ T cells in 41% of UC-patients.

Immune status of UC-patients have been significantly improved after treatment with Gepon. Particularly, in 67% of patients, counts of perforin-positive cytolytic NK cells were increased after treatment with Gepon in otherwise exhausted total population of NK cells. The population of cytolytic CD8$^+$ T cells with perforin, also underwent a correction as a result of treatment with Gepon. After treatment, there was a decrease in the pathologically enhanced counts of perforin-positive cytolytic CD8$^+$ T cells in all the UC-patients who initially had above 100 cytolytic CD8$^+$ T cells per 1 µl of the blood, or above 25% cytolytic CD8$^+$ T cells among the total population of CD8$^+$ T cells. In the patients with an initially normal content of cytolytic CD8$^+$ T cells with perforin, the number of these cells did not change after treatment.

CONCLUSION

1. The application of Gepon for the treatment of UC succeeded in producing clinico-endoscopic improvement and overcame the resistance to basic anti-inflammatory therapy in 30 of 36 (83.3%) patients.

2. After treatment by Gepon, there were substantial increases in the number of perforin-positive cytolytic NK cells within the exhausted total population of NK cells. On the contrary, the content of pathologically elevated cytolytic CD8$^+$ T cells was reduced after treatment with Gepon to levels approaching normal values.

3. The preparation of immuno-enhancer Gepon is recommended for treatment of the chronic distal forms of UC (proctitis, proctosigmoiditis) as part of combination therapy for this disease.

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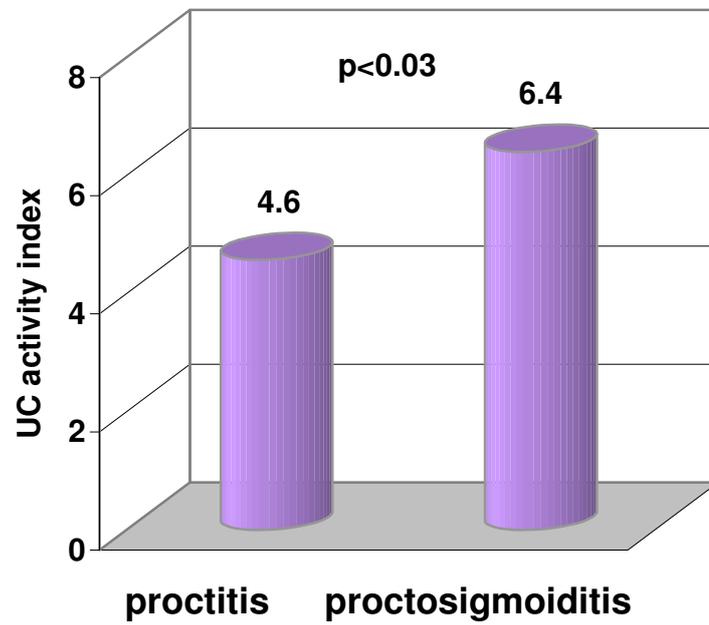
Figure 1. Index of gravity of non-specific ulcerative colitis in patients with proctitis and patients with proctosigmoiditis
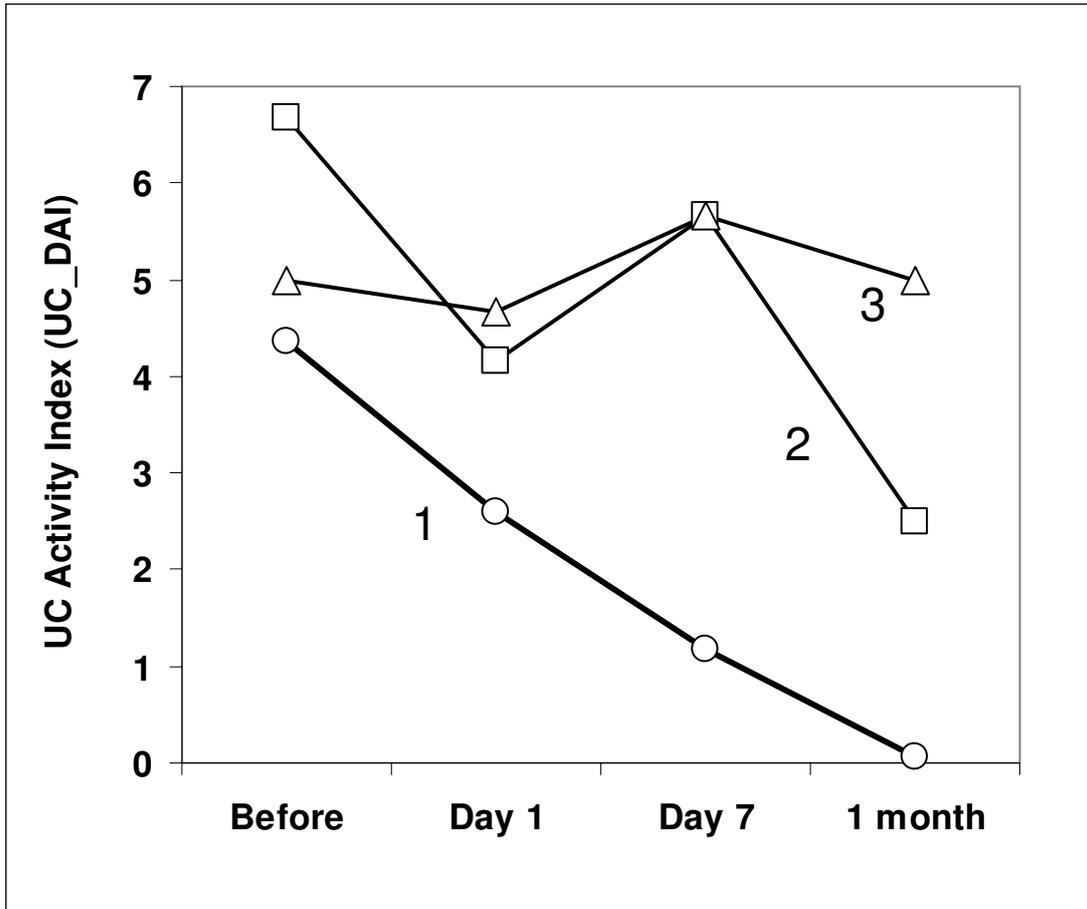
Figure 2. Change in the gravity of non-specific ulcerative colitis in the patients with proctitis after treatment with Gepon: 'response 1' good effect (65% of cases); 'response 2' satisfactory effect (27% of cases); 'response 3' no effect (8% of cases)
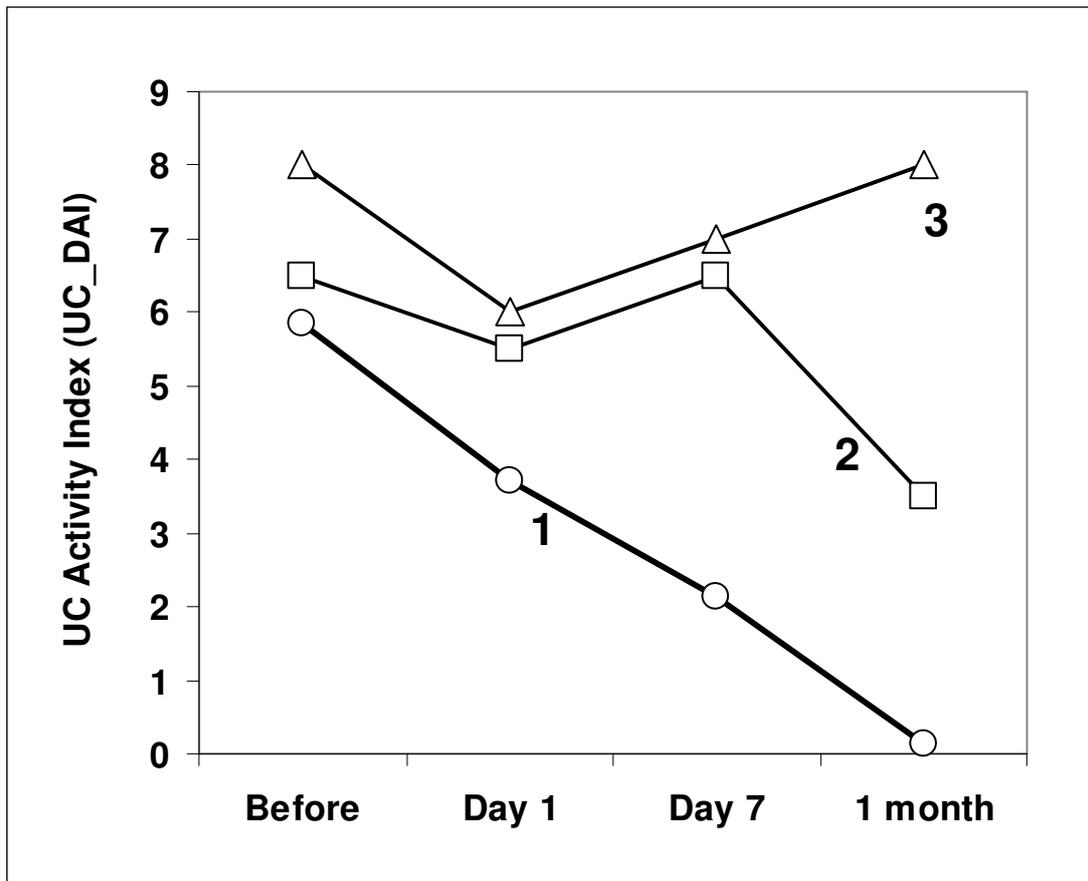
Figure 3. Change in the gravity of non-specific ulcerative colitis in patients with the proctosigmoiditis after treatment with Gepon: 'response 1', a good effect (70% of cases); 'response 2' satisfactory effect (20% of cases); 'response 3' no effect (10% of cases)

Table 1. Dynamics of the UC activity index in response to rectal treatment with Gepon suppository in patients of Groups 1 and 2.

	Patients of Group-1 and Group 2 (n = 26)	UC Activity Index (M $\pm$ m)
1	before treatment	4.96 $\pm$ 0.2
2	1st day of treatment	3.19$\pm$ 0.2
3	7th day of the treatment	2.73 $\pm$ 0.3
4	1 month after the treatment	1.19 $\pm$ 0.3

Note: $p_{1-2} < 0.001$, $p_{1-3} < 0.001$, $p_{1-4} < 0.001$, $p_{2-3} > 0.05$, $p_{2-4} < 0.001$, $p_{3-4} < 0.001$.

Table 2. Dynamics of the UC activity index in response to rectal treatment with Gepon micro-enemas in patients of Group-3.

	Patients of Group-3 (n = 10)	UC Activity Index (M $\pm$ m)
1	before treatment	6.2 $\pm$ 0.32
2	1st day of treatment	4.3 $\pm$ 0.54
3	7th day of the treatment	3.5 $\pm$ 0.75
4	1st month after the treatment	1.6 $\pm$ 0.86

Note: $p_{1-2} < 0.01$, $p_{1-3} < 0.002$, $p_{1-4} < 0.001$, $p_{2-3} > 0.05$, $p_{2-4} < 0.01$, $p_{3-4} < 0.05$.