Kharkov National Medical University

Internal Medicine Department No. 3

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REPORT:

EFFICACY OF FULLERENE (C_{60}) APPLICATION IN COMPLEX THERAPY OF PATIENTS WITH ULCERATIVE DISEASE

(English Version of Report after Russian Manuscript Draft Translation)

Kharkov – 2009

ХАРЬКОВСКИЙ НАЦИОНАЛЬНЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

1. **Кафедра внутренней медицины №3**

«УТВЕРЖДАЮ»
Проректор по научной работе
профессор В.В. Мясоедов
_____
_____ 2009г.

ОТЧЕТ

ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ ФУЛЛЕРЕНА В КОМПЛЕКСНОЙ ТЕРАПИИ БОЛЬНЫХ ЯЗВЕННОЙ БОЛЕЗНЬЮ

Харьков - 2009

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CONTENTS

URGENCY

Peptic ulcer (PU) is the most significant issue of healthcare [3, 8, 9, 18, 19, 22, 33]. It belongs to most spread diseases, affecting young and middle-aged people, often conditioning temporary and frequently stable loss of working capacity. Although many issues of PU etiology and pathogenesis are still unsolved, in recent years new data have been obtained, which to a large extent explain the mechanism of the disease. Discovery of a new bacterium species – Helicobacter pylori (H. pylori) in 1983 was of great importance. Numerous investigations proved participation of H. pylori in PU genesis, especially at duodenal ulcer (DU), the mechanisms of ulcerogenic action of Helicobacter infection were decoded [3, 17, 19, 36, 37, 40]. Works of numerous authors convincingly prove that DU is accompanied with suppression of both cellular and humoral components of the immune system [3, 18, 23, 28, 30]. Decrease of local immune protection increases susceptibility to the action of H. pylori, which has antigenicity identical to normal mucous. This peculiarity induces autoimmune processes, leading to both death of H. pylori, and mucous membrane lesion. Thus, there occurs a situation “allograft against master”. Tissue hypoxia, which develops as a result of autoimmune inflammation, is accompanied with activation of lipid peroxidation (LPO) system and decrease of the functional state of antioxidative protection (AOP) [4, 7, 10, 14, 21]. Possibly, it is under the influence of these processes that duodenal ulcerogenesis develops and manifests via duodenal ulcers.

The determined bond of H. pylori with PU allowed the generation of a new approach to treatment and prevention of this disease. Conventional treatment of DU is aimed at elimination of H. pylori [1, 6, 11, 27, 31, 38, 39]. However, taking into consideration other factors, apart from H. pylori, which participate in the development of the disease, even full eradication of this microorganism does not solve the whole issue of PU treatment. Medicinal agents, influencing different pathogenetic components of the diseases, preserve their significance. At the same time, the issue of correction of LPO processes and immune protection in this patient population is still practically undeveloped.

Biologically active supplement fullerene (in abbr.form, BAS or fullerene), which had already undergone preclinical study, demonstrated the absence of acute and subchronic toxicity at its use [26, 32, 34, 35]. There were also revealed numerous specific activities of fullerene in animal studies, including anti-inflammatory, antiulcer, regenerative, membrane stabilizing, adaptogenic, which can be the basis for development of a new therapeutic strategy at use of conventional therapy of PU in combination with fullerene. Possibly, the indicated approach of treatment of PU patients can improve the effectiveness of the therapy of this patient category.

Objective of the study. Optimization of the treatment of duodenal ulcer patients at use of conventional therapy in combination with fullerene.

Main tasks of the study.

1. To determine the influence of combined treatment with fullerene on clinical manifestations of DU.
2. To study the indexes of clinical blood analysis in the examined PU patients under the influence of the proposed therapy with fullerene in comparison with conventional therapy.
3. To study the state of acid-forming function of the stomach in DU patients at use of conventional treatment in combination with fullerene.
4. To determine the endoscopic criteria of the state of duodenal mucous coat with subsequent biopsy of the mucous in DU patients at use of the proposed therapy with fullerene.
5. To determine the degree of eradication of Helicobacter infection in DU patients at use of combined treatment with fullerene.
6. To study the effectiveness of influence of the treatment with fullerene in combination with conventional therapy on the state of LPO system and AOP in DU patients.
7. To determine the state of protein exchange in DU patients at use of conventional therapy in combination with fullerene.
8. To determine the state of the immune system in DU patients at use of the proposed therapy with fullerene.

MATERIALS AND METHODS OF THE STUDY

1.1. Clinical characteristics of the examined patients

There were examined 40 patients, undergoing inpatient treatment at the gastroenterological department of Municipal health care institution “Kharkov regional clinical hospital – Centre of emergency medical care and disaster medicine”, which is a base of Internal medicine department No. 3 of KhNMU.

All patients were diagnosed with DU. Then the patients were subdivided into 2 clinical groups due to the method of treatment. Group 1 (20 individuals) – patients, receiving conventional therapy, group 2 (20 individuals) – receiving proposed therapy (combining conventional with BAS fullerene). The control group consisted of 20 practically healthy individuals.

The diagnosis DU was formulated according to the unified clinical statistical classification of the diseases of digestive organs (Kiev, 2004). Forming and verification of the diagnosis was based on the results of complex clinical laboratory and instrumental examination in accordance with the Order of the Ministry of Health of Ukraine No. 271 d/d 13.06.2005, which includes the analysis of acid-forming function of the stomach (intragastric pH-metry), fibrogastroduodenoscopy with biopsy of duodenal mucous coat and determination of H. pylori contamination degree. All studied parameters were determined for 14 days in the dynamics of treatment.

All examined patients were 19 – 68 years old. Females, aged 19 – 44, prevailed – 29 individuals (73%).

All examined patients had clinical characteristics, which can be considered to be the manifestations of abdominal painful, dyspeptic, dyskinetic and asthenovegetative syndromes.

The main syndrome in DU patients (100% cases) was abdominal painful, characterized by nagging, dull pains in epigastrium and/or in the right hypochondrium of moderate or expressed intensity. The pains were clearly

interconnected with food intake: occurred on the empty stomach. Painful syndrome had stable persistent character. After single intake or antiulcerous therapy for several days the pains disappeared and renewed again after discontinuation of the preparations.

DU patients had complains about feeling of heaviness and overfilling of the stomach after meals (73%), air eructation, acid eructation. More than half the patients (78%) were disturbed by heartburn, sometimes the patients noted foul taste in the mouth, abdominal distension, borborygmus, nausea, vomiting. Many patients (56%) were constantly disturbed by dyspeptic phenomena, which increased after nutritional inaccuracy (heavy meal, spicy, smoked food). Some patients (5%) noted increase of dyspeptic symptoms at emotional overload. In 73% of the patients there were observed indirect signs of gastrocecal and duodenogastric refluxes.

Asthenovegetative disorders in DU patients (98%) had functional character. The patients complained about general weakness, rapid fatigability, decreased working capacity, headaches, increased irritability and mood lability.

In order to make a more precise diagnosis of DU all patients underwent complex clinical laboratory and instrumental examination.

1.2. Methods of patient examination

In order to achieve the objective and solve the tasks of the study there were conducted clinical and laboratory examinations, including the study of indexes of clinical blood analysis, determination of acid-forming function of the stomach, endosonography of duodenum with target biopsy of duodenal mucous coat and further determination of the degree of H. Pylori contamination, as well as the study of indexes of protein exchange, LPO and AOP systems, immune status in DU patients in the dynamics of conventional and proposed therapies, which allow to estimate the functional state of duodenum in these patients.

In the examined patients there was used a method of basal topographic intragastric pH-metry with the use of special probes PE-pH-2 [12, 20, 29]. According to the estimated indexes the acid-forming function of the stomach was characterized

as hypo-, normo- and hyperacid. The results were registered on acidogastrograph AG 1D-01.

All patients underwent gastrofibroduodenoscopy with flexible gastroscope “GD-B-BO-4” produced by the firm “LOMO” with further target biopsy of the mucous coat of the stomach and duodenum [5, 25]. In the process of the study there was carried out macroscopic estimation of the state of the mucous coat of the esophagus, stomach and duodenum in accordance to the criteria of the European Society of Gastrointestinal Endoscopy (2000).

To achieve reliable results, the biopsy was carried out from 3–4 areas of the mucous (fundus, body, antrum, pyloric canal of the stomach and duodenal cap). Biopsy material was preserved in 10% neutral formalin after alcohol dipping and embedded into paraffin. Sections of 5–6 μm thickness were stained according to Romanovsky-Giemsa (H. pylori identification). Microbial count in biopsy material was carried pit under immersion microscope in 30 fields [17]. The estimation was carried out on the following criteria: 0 – no bacteria in the preparation, 1 – mild contamination (up to 20 microbial bodies in the field), 2 – moderate contamination (20 – 50 microbial bodies in the field), 3 – expressed contamination (over 50 microbial bodies in the field).

For LPO estimation there was used the method of serum biochemiluminescence count, based on registration of electromagnetic optical range radiation with chemiluminometer CLM1C-01 [10]. The following indexes were determined: SCL, CL Fe^{2+}, (light-sum and scintillation) and CL H_2O_2, (light-sum and scintillation). The state of the system of antioxidative protection was estimated on the basis of peroxidative activity according to the method of T.P. Popov and L.P. Neykova and according to the determination of blood catalase by the method of Bach [24]. In order to determine SH-groups of protein and nonprotein compounds there was used photocolorimetric assay ultramicromethod by V.F. Folomeyev.

There was determined the state of protein exchange in the examined patients – the level of whole serum protein (biuret test), serum protein fractions (electrophoretic method on paper) [2, 13].

To determine the number of T- and B-lymphocytes and subpopulations of T-lymphocytes there was carried out the reaction of indirect immunofluorescence with the use of monoclonal antibodies. With the use of monoclonal antibodies LT-1 (OCT-1) there were detected antigens (CD5), typical for population of T- lymphocytes; with antibodies LT-4 (OCT-4) – antigenic marker of subpopulation of T- helpers/inductors (CD4), with LT-8 (OCT-8) – markers of T- suppressors/ cytotoxic lymphocytes (CD8). Monoclonal antibodies 3F3 were used to detect surface membrane antigen of B-lymphocytes. The determination of immunoglobulins was carried out by the method in Manchini modification, with the use of apparatus “Reagent”.

Statistical analysis of the obtained data was carried out with the use of parametric (derived quantity relative and average, estimated probability with the use of Student’s t-criterion) methods [16]. A program package, adapted for medical and biological investigations, was used in the work [15].

All DU patients were subdivided into two groups: group 1 (20 individuals) – patients, receiving conventional therapy, group 2 (20 individuals) – patients, receiving proposed therapy.

Conventional therapy for DU included along with individual diet a complex of “triple” anti-helicobacter therapy (in Hp-positive cases) for 7 days: Rabeprazole (Pariet) – 40 mg a day, Amoxicillin – 1000 mg twice a day, Clarithromycin – 500 mg twice a day with further use of antisecretory agents for two weeks. In case of Hp-negative ulcers the basic strategy of the treatment was indication of Rabeprazole for 3–4 weeks.

The proposed therapy of DU included additional use along with conventional therapy of BAS fullerene – 50 ml 15-20 min before meals 3 times a day for the first 3 days, twice a day for the following 3 days, once a day for the following 15 days.

RESULTS OF THE STUDY AND THEIR DISCUSSION

2.1. Comparative characteristic of the influence of conventional and proposed therapies on clinical manifestation of peptic ulcer in the examined patients

Table 2.1

Frequency of the main clinical syndromes in DU patients in the dynamics of treatment

Clinical syndromes	Groups of the examined patients					
	DU before treatment, n=40		1 (conventional therapy), n=20		2 (combined therapy with fullerene), n=20	
	Abs.	%	Abs.	%	Abs.	%
Abdominal painful	40	100	3	15	-	-
Dyspeptic	31	78	3	15	-	-
Dyskinetic	29	73	3	15	1	5
Asthenovegetative	39	98	4	20	1	5

At the analysis of clinical indexes in the examined patients of group 1, receiving conventional therapy, there was observed subjective improvement and tendency to normalization of objective data as decreased degree of expressiveness of abdominal painful (85%), dyspeptic (85%), dyskinetic (85%) and asthenovegetative (80%) syndromes by day 7-8 of the hospital (Tabl. 2.1).

The proposed method of treatment, used in group 2 of DU patients, provided clear positive effect: abdominal painful (100%), dyspeptic (100%), dyskinetic (95%) and asthenovegetative (95%) syndromes eliminated by day 5-7 of the initiation of the therapy.

In general case the use of fullerene had additional to conventional therapy optimizing influence on the course of the inflammatory process. The

improvement of clinical symptomatics in DU patients against the background of different variants of treatment was a logical result. The use of fullerene promoted rapid and final evolution of all complexes of clinical manifestations of duodenal ulcers. Possibly, it was connected with activation of natural sanogenetic mechanisms in the patients of the main group. Consequently, the use of combined therapy with fullerene had more rapid and expressed action on positive dynamics of clinical manifestations in DU patients, than conventional treatment.

2.2. Indexes of clinical blood analysis in duodenal ulcer patients under the influence of conventional and proposed therapy with fullerene

Table 2.2

Indexes of clinical blood analysis in the examined patients

Index	Groups of the examined patients			
	Control, n=20	DU before treatment, n=40	1 (conventional therapy), n=20	2 (combined therapy with fullerene), n=20
Hemoglobin, g/l	129.7±5.3	127.6±3.3*	129.5±5.1	129.6±5.1*/**
Erythrocytes, 10^{12}/l	4.1±0.2	3.9±0.1*	3.95±0.2	4.0±0.1*/**
CP	0.91±0.02	0.85±0.01*	0.89±0.02	0.91±0.01*/**
ESR, mm/h	6.2±0.2	17.1±0.1*	15.1±0.1	10.2±0.2*/**
Leukocytes, 10^{9}/l	4.7±0.8	11.1±0.7*	10.2±0.7	7.2±0.6*/**
Stab, %	4.8±0.5	7.1±0.4*	6.2±0.4	5.1±0.4*/**
Segmental, %	51.2±1.3	71.4±1.1*	65.0±1.3	55.4±1.2*/**
Eosinophils, %	0.6±0.2	0.8±0.1*	0.8±0.1	0.6±0.1*/**
Lymphocytes, %	21.4±1.1	38.1±1.0*	31.2±1.1	22.5±1.1*/**
Monocytes, %	3.6±0.5	4.1±0.4*	4.0±0.5	3.7±0.5*/**

Basophiles, %	0.2±0.2	0.4±0.1*	0.4±0.2	0.3±0.1*/**

Note. p<0.05; *significant in comparison with control;
** in comparison with identical indexes in the patients of group 1 and group 2.

In 6 DU patients (15%) there was observed a tendency to leukocytosis and accelerated ESR, which reflected the presence of inflammatory processes in the mucous coat of the gastroduodenal zone in this patient population. Indicated changes of hemogram could be the manifestations of immune inflammatory processes, and also serve as markers of endogenous intoxication. Under the influence of the conducted treatment in the patients of group 1 there was observed a tendency to decrease of inflammation signs by day 7 of the hospital. In the patients of group 2, receiving conventional therapy in combination with fullerene, there were no signs of inflammatory processes in clinical blood analysis by day 5 of the treatment. It is necessary to note, that positive dynamics of hemogram indexes in group 2 patients was conditioned by the expressed immunotropic effect of fullerene, and also demonstrated no toxic influence on the system of blood-formation.

Thus, additional indication of fullerene to conventional therapy in DU patients had positive influence on the indexes of clinical blood analysis, which can be explained by powerful anti-inflammatory and immunotropic influence of this medicinal agent.

2.3. The state of acid-forming stomach function in duodenal ulcer patients under the influence of conventional therapy in combination with fullerene

Table 2.3

pH indexes in basal intermediary part of the stomach in the examined patients

Group of patients	The level of basal parietal pH					
	hyperacidity		normoacidity		hypoacidity	
	abs.	%	abs.	%	abs.	%
DU before treatment, n=40	36	90	-	-	4	10
1 (conventional therapy), n=20	3	15	18	90	2	10
2 (combined therapy with fullerene), n=20	-	-	20	100	-	-

Practically in all examined DU patients there were dominating processes of increased secretion (pH=1.21±0.1), leading to hyperacidity and ulcerogenesis.

On day 10 of the conducted treatment in group 1 patients, receiving conventional therapy, normoacidity was observed in 18 patients, which constituted 90%. In the patients of group 2, receiving combined therapy with fullerene, the state of acid-forming normalized in 100% patients.

It is possible to assume, that indication of the preparation fullerene had complex positive influence on the state of gastroduodenal system, which was realized on cellular and subcellular levels. Restoration of the accessory cell functioning demonstrated the activation of genetically determinated programme of reparative regeneration, which restored morpho-functional unity and structural adequacy of digestive organs. Probably, fullerene has powerful regenerative, wound-healing and bactericidal properties. In addition, it is possible to assume the presence of systemic effects of fullerene use, which realize their influence providing optimal mechanisms of neurovegetative regulation of the secretory function of the stomach.

The study of acid-forming function of the stomach in DU patients in the dynamics of different treatment regimens testifies to the fact, that in the patients of group 2, receiving complex therapy with fullerene, there is observed more expressed normalization of acid-forming against the patients of group 1, receiving conventional treatment. Thus, indication of fullerene was a pathogenetically conditioned and highly effective method of the correction of acid-forming function of the stomach in DU patients.

2.4. Effectiveness of the influence of conventional therapy in combination with fullerene on endoscopic criteria of duodenal mucous coat in ulcer patients

At duodenal endoscopy of DU patients there was estimated the presence of changes of superficial duodenitis type in combination with single ulcers in the duodenal bulb (Tabl. 2.4).

Table 2.4

Indexes of duodenal endoscopy study in the examined patients

Changes of the duodenal mucous coat	Groups of the examined patients					
	DU before treatment, n=40		1, n=20		2, n=20	
	abs.	%	Abs.	%	abs.	%
Duodenopathy: erythematous (hyperaemia)	20	50	7	35	-	-
erosive	–	–	–	–	-	-
hemorrhagic	–	–	–	–	-	-
congested	10	25	2	10	-	-
Superficial duodenitis	21	53	-	1	-	5
Erosions of duodenal bulb	–	–	–	–	-	-
single ulcers in duodenal bulb in: - fibrin phase	32	80	2	10	-	-
- granulation phase	7	18	18	90	15	75
- scar phase	1	3	-	-	5	25

Note. group 1 – patients, receiving conventional therapy;
group 2 – patients, receiving combined therapy with fullerene

The ulcers were located on the anterior, posterior, basal, anterosuperior and anteroinferior walls of the duodenal bulb at approximately similar number of

observations in both groups. And 32 (80%) ulcers in DU patients were in fibrin phase. Their sizes varied within 0.5 – 1.0 cm. In 6 cases (15%) there were ulcers in the phase of early granulations, in 1 case (3%) – in the phase of mature granulations and in one observation (3%) – in the phase of red stellar scar.

In 14 days under the influence of conventional therapy in group 1 DU patients there was observed decrease of inflammatory processes in the duodenal mucous coat in 13 patients, which constituted 65%, congested processes and acceleration of the granulation processes in 18 patients, which constituted 90%.

In group 2 DU patients at the use of combined therapy with fullerene the absence of inflammatory processes in the duodenal mucous coat was observed practically in all examined patients (19 individuals), which constituted 95%, congested processes – in 100% patients, acceleration of the granulation and scarring processes – in all patients.

Conducted studies enable to conclude, that indication of fullerene provided the process of rapid and adequate regeneration in the zone of ulcer. The indicated effect is conditioned both optimization of microcirculation and restoration of tissue metabolism and improvement of the processes of immune supervision over cellular proliferation. Stability of exchange metabolic processes, leading to adequate ulcer healing, was also interconnected with activation of natural desintoxic and drainage systems of the organism (first of all, mucous coat of gastrointestinal tract), decreased activity and complete completion of the inflammatory process. Thus, it was established, that the presence of anti-inflammatory and regenerative properties of fullerene, manifested in cooperation of immune, metabolic and microcirculatory reactions of the organism and aimed at healing of duodenal ulcers.

2.5. The study of Helicobacter infection in duodenal ulcer patients at use of conventional therapy in combination with fullerene

H. pylori was observed in most examined DU patients (Tabl. 2.5).

On day 14 of the conducted treatment under the influence of conventional

therapy practically in all patients of group 1 there was observed eradication of Helicobacter infection (90%). However in the patients of group 2, additionally receiving fullerene, eradication of H. pylori was observed in 100% patients.

Table 2.5

Indexes of histological study biopsy material in the duodenal mucous coat in the examined patients

Degree of intensity of H. pylori	Groups of the examined patients					
	DU before treatment, n=40		1, n=20		2, n=20	
	abs.	%	abs.	%	abs.	%
0	2	5	18	90	20	1
1	5	13	2	10	-	-
2	13	33	-	-	-	-
3	20	50	-	-	-	-

Note. group 1 – patients, receiving conventional therapy;
group 2 – patients, receiving combined therapy with fullerene.

Thus, H. pylori initially infects epithelium cells of the mucous coat of the stomach and duodenum. Having similar to epithelial cell antigenicity, the microorganism develops autoimmune reaction to the mucous coat of the stomach and duodenum. Along with the death together with epithelial cells, H. pylori exists in the mucus between folds of the mucous coat or penetrates into deep tissue layers of the duodenum. It promotes the processes of ulcerogenesis. That is why the contamination of the mucous coats of the stomach and duodenum conditions the severity of DU patient lesions.

Fullerene had expressed antimicrobial activity against H. pylori in the patients of the main group. It is possible to assume its direct bactericidal properties, as well as stimulation influence on macrophage function. As one of the main problems of antibacterial therapy is polychemoresistance of microorganisms, it is possible to assume, that fullerene is able to destroy protective structural barriers and promotes activation of phagocytal reaction. In addition, indication of fullerene increases susceptibility of H. pylori to the conventional treatment. The most significant effect of fullerene in DU patients one may consider the optimization of eradication therapy.

2.6. The state of lipid peroxidation and antioxidative protection system in duodenal ulcer patients at use of conventional therapy in combination with fullerene

Conducted studies enabled to reveal significant differences in the indexes of LPO and AOP in the examined patients under the influence of different treatment regimens (Tabl. 2.6, 2.7).

The study of serum antioxidative state by the method of biochemiluminescence demonstrated the decrease of serum antioxidative potential in DU patients in comparison with control group. Tissue hypoxia, as an integral part of PU pathogenesis, led to damaging action of LPO products on the mucous coat of the duodenum. Decompensation of adaptive homeostatic reaction from the side of AOP manifested in atrophic processes against the background of chronic inflammation, and then in development of ulcerous defects.

Table 2.6

Indexes of biochemiluminescence in DU patients in the dynamics of treatment

Groups of the examined patients				
Index	control, n=20	DU before treatment, n=40	1 (conventional therapy), n=20	2 (combined with fullerene), n=20
SCL, count/sec	28±2	85 ± 2*	61 ± 4	29 ± 2*/**
CL H_2O_2, count/sec, scintillation	2970±50	4560±80*	3720±70	2988±65*/**
CL H_2O_2, count/sec, light-sum	1176±30	2030 ± 50*	1630 ± 50	1195 ± 40*/**
CL Fe^{2+}, count/sec, scintillation	606±18	820 ± 14*	760 ± 25	614 ± 20*/**
CL Fe^{2+}, count/sec, light-sum	91±4	195 ± 8*	150 ± 9	95 ± 5*/**

Note. $p<0.05$; *the difference is statistically significant in comparison with control group. ** in comparison with identical indexes in the patients of group 1 and group 2.

Table 2.7

Indexes of AOP (M±m) in DU patients in the dynamics of treatment

Index	Groups of the examined patients			
	control, n=20	DU before treatment, n=40	1 (conventional therapy), n=20	2 (combined with fullerene), n=20
Peroxidase, μmol/minЧl	282.0±5.41	227.7 ± 3.22*	253.0 ± 0.82	255.0±3.17*/**
Catalase, mg	16.5±0.34	13.3 ± 0.18*	15.1 ± 0.17	15.4 ± 0.17*/**
SH-groups total, mmol/l	2.43±0.05	1.21 ± 0.11*	2.18 ± 0.15	2.27 ± 0.14*/**
Nonprotein SH-groups, mmol/l	0.85±0.02	0.51 ± 0.05*	0.68 ± 0.06	0.77 ± 0.06*/**
Protein SH-groups, mmol/l	1.56±0.06	0.70 ± 0.05*	1.45 ± 0.09	1.59 ± 0.08*/**

Note. p<0.05; *significant in comparison with control.
** in comparison with identical indexes in the patients of group 1 and group 2.

Serum concentration of LPO products differed significantly under the influence of treatment from initial values only in group 2 (Tabl. 2.6). Possibly, the proposed method of treatment promotes decrease of LPO activity in the serum due to powerful antioxidative effect of fullerene, manifested in increased activity of reduction-oxidation processes.

Concentration of peroxidase and catalase under the influence of different regimens of treatment differed significantly between the groups of DU patients. Consequently, antioxidative enzymes are the most mobile component of the protection against oxidative stress at DU. Increased activity of these enzymes stimulates reparative processes, necessary to provide functional unity of the organism. Concentration of SH-groups and their fractions increased significantly in the groups, having significant differences of the level of nonprotein sulfhydric compounds between the groups.

That is why complex treatment of DU patients with fullerene in group 2 was a more effective method of oxidative status correction, than conventional therapy. Antioxidative activity of fullerene was conditioned by inhibition of generation of reactive oxygen forms (singlet oxygen and superoxideradical), because stable

hydrated coat did not allow the molecule of oxygen to fix on the surface of nanosoma. It provided stability of water-containing cellular structures and cellular exchange. Fullerene manifested “reasonable” and long-term antioxidative activity owing to regulation of free radical level in the focus of inflammation. It is this peculiarity that differs fullerene from other known antioxidants.

The studies estimated the activation of LPO system and inhibition of AOP in DU patients. Under the influence of the conducted treatment there was observed more expressed improvement of antioxidative processes in DU patients, receiving combined treatment with fullerene. As the state of LPO and AOP is one of the key components of homeostasis of the human organism, there were estimated significant possibilities of fullerene in this patient population.

2.7. The state of protein exchange in duodenal ulcer patients at use of conventional therapy in combination with fullerene

Indexes of protein exchange in the examined patients indicated the difference in its state in DU patients before treatment in comparison with control group and between clinical groups 1 and 2 (Tabl. 2.8).

Table 2.8

Indexes of protein (M±m) in DU patients in the dynamics of treatment

Index	Examined groups			
	control, n=20	DU before treatment, n=40	1 (conventional therapy), n=20	2 (combined with fullerene), n=20
Whole protein, g/l	69.5 ± 1.75	62.8 ± 1.15*	64.3 ± 0.44	65.7 ± 0.42*/**
Albumins, %	57.5 ± 0.52	53.6 ± 0.62*	55.8 ± 0.36	56.0 ± 0.41*/**
Globulins, %: alpha-1	5.24 ± 0.12	5.72 ± 0.15*	5.48 ± 0.14	5.32 ± 0.28*/**
alpha -2	9.39 ± 0.15	9.44 ± 0.27*	9.42 ± 0.25	9.40 ± 0.36*/**
beta	12.0 ± 0.18	13.0 ± 0.34*	12.5 ± 0.31	11.7 ± 0.28*/**
gamma	15.8 ± 0.33	18.3 ± 0.35*	16.9 ± 0.22	15.2± 0.28*/**

Note. $p<0.05$; *significant in comparison with control group.
** in comparison with identical indexes in the patients of group 1 and group 2.

Whole protein level was decreased in all DU subjects in comparison with control. And the differences were in changes of the content of albumin and gamma-globulin fraction of blood protein spectrum of the examined patients. Decreased concentration of albumins in the conducted study can be explained by disturbed absorption of amino acids, secondary changes of protein synthesis in the liver. Increase of gamma-globulin fraction can be considered as a result of inflammatory changes of the duodenal mucous coat in the examined patients.

Significant improvement of protein exchange indexes was registered both against the background of conventional and proposed complex therapy (Tabl. 2.8). Positive changes in proteinogram were observed in group 1 only by the end of week 2, and in group 2 – at the end of week 1 or beginning of week 2 of the treatment. In addition there was a more expressed improvement of protein exchange in group 2 due to increased concentration of whole protein, albumins and decreased level of gamma-globulins, than in DU patients, receiving conventional therapy.

Probably, fullerene not only provides increased metabolic potential of the organism due to improved absorption of amino acids in duodenum, but also restores functional properties of hepatocytes. Increased level of whole protein and normalization of fractional content in the patients of group 2 was also conditioned by protection against proteolytic “attack” against the background of the inflammatory process. The latter once again proves the powerful antioxidative potential of fullerene.

2.8. The state of immune protection in duodenal ulcer patients at use of conventional therapy in combination with fullerene

During conducted immunological studies there were estimated significant differences in the indexes of cellular and humoral components of the immunity in the examined population under the influence of different regimens of treatment (Tabl. 2.9).

Table 2.9

Indexes of cellular and humoral immunity (M±m) in DU patients in the dynamics of treatment

Index	Groups of the examined patients			
	control, n=20	DU before treatment, n=40	1 (conventional therapy), n=20	2 (combined with fullerene), n=20
T-lymphocytes (CD3), %	68.0±2.72	56.5 ± 0.93*	65.7 ± 0.72	66.8 ± 0.42*/**
B-lymphocytes (CD22), %	8.0±0.5	6.62 ± 0.20*	7.32 ± 0.22	7.75 ± 0.23*/**
O-lymphocytes (NK-cells-CD16), %	12.0±1.0	36.8 ± 1.14*	26.7 ± 0.70	25.4 ± 0.45*/**
T-helpers (CD4), %	39.0±1.83	46.4 ± 0.58*	42.3 ± 0.44	41.7 ± 0.43*/**
T-suppressors (CD8), %	23.0±0.94	18.3 ± 0.37*	19.8 ± 0.33	21.9 ± 0.32*/**
T- helpers / T- suppressors	1.68±0.13	2.53 ± 0.15*	2.14 ± 0.12	1.90 ± 0.10*/**
Ig, mg/ml A	1.9±0.16	2.82 ± 0.18*	2.52 ± 0.11	2.38 ± 0.12*/**
G	11.7±0.36	17.7 ± 0.43*	15.3 ± 0.33	14.2 ± 0.31*/**
M	1.25±0.12	2.05 ± 0.15*	1.48 ± 0.12	1.41 ± 0.11*/**

Note. $p<0.05$; *significant in comparison with control.
** in comparison with identical indexes in the patients of group 1 and group 2.

The number of T-lymphocytes differed significantly in DU patients in comparison with control group. This fact indicates inhibition of T-cellular components of the immunity, especially expressed in DU patients.

Weakening of the immune surveillance could be associated with activation of LPO processes, damage of immunocyte membranes, as well as immunosuppressive influence of structural protein decay products against the background of tissue hypoxia. There was observed statistically significant increase in the number of T-helpers in all examined DU patients in comparison with control group. At the same time there was registered significant decrease of T-suppressors. It led to increased values of immuneregulatory index (T-helpers/T-suppressors) in the examined patients (Tabl. 2.9). In addition, there was established increased concentration of

immunoglobulins of A, G and M classes in all examined DU patients in comparison with control, which reflected activation of humoral immunity.

Under the influence of the conducted treatment decrease in the number of T-helpers and increase of T-suppressors, more expressed in group 2 patients, led to significant decrease of immuneregulatory index in comparison with group 1 DU patients (Tabl. 2.9). This fact demonstrates the increase of activity of proliferative processes against the background of decreased autoimmune aggression and improved recognition of own antigen determinants of the patient organism. Content of T-lymphocytes and NK-cells significantly changed in all patients. Consequently, in all patients there was registered definite anti-inflammatory effect of the therapy. On the contrary, expressed change of the level of B-lymphocytes was observed only in group 2 against the background of the proposed therapy with fullerene, which can be explained by decrease of autoimmune aggression. It is possible to assume, that in addition to elimination of infectious origin antigen, there also occurred increase of own immunereactivity in DU patients against the background of fullerene use.

From the side of humoral component of the immunity in the patients of group 2 there was registered significant decrease of concentration of A, G and M class immunoglobulins. Concentration of IgG significantly decreased only in group 2 patients, which is explained by decreased activity of the infectious process. Normalization of cellular immunity indexes demonstrated the peculiarities of immunotropic effect of fullerene, which provides elimination both general and local manifestations of the inflammatory process. It is possible to assume positive influence on the state of intestinal biotope, taking part in the proliferation of immune cells via lipid A and Kupffer cells. Providing of effective humoral immune protection was associated both with completion of autoimmune reactions and restoration of natural barrier properties of the intestinal mucous. Probably, the latter effect was interconnected with restoration of drainage properties of the mucous coat at the glycocalix level.

The results of complex therapy with fullerene in DU patients, given in the work, allow to assume several possible ways of immunocorrection.

First of all, antihelicobacter therapy was accompanied with decrease of systemic stimulation of autoimmune processes. In addition, immunotropic effect of the treatment with fullerene was accompanied with increase of immunoreactivity level due to increase of B-lymphocyte content and decrease of NK-cells. Anti-inflammatory effect of the treatment, which was accompanied with inhibition of prostaglandin synthesis, also decreased the activity of NK-cells. It is possible to think, that decrease of T-helper level and increase of T-suppressor level stimulated reparative processes of proliferation of duodenal mucous epithelium. The change of immunoglobulins level was connected with H. pylori elimination, decrease of inflammatory phenomena and normalization of the processes of own antigen recognition. That is, the proposed method of complex therapy with fullerene promoted the reparation of cell identification of “own – foreign” type and inhibition of autoimmune reaction “recipient against master”.

The estimated peculiarities enable to think that the proposed complex of combined conventional therapy with fullerene significantly improves the state of the immune system in DU patients. Therefore, use of BAS fullerene in addition to conventional therapy is a more effective complex of treatment in comparison with the treatment, conducted in the group of comparison.

CONCLUSIONS

1. Additional to conventional therapy indication of fullerene promotes more rapid elimination of clinical manifestations of abdominal painful, dyspeptic, dyskinetic and asthenovegetative syndromes in DU patients.
2. Use of fullerene promotes the normalization of indexes of clinical blood analysis in DU patients, which was associated with expressed anti-inflammatory effect of treatment and absence of toxic influence of the medicinal agent.
3. Inclusion of BAS fullerene in complex treatment of DU patients promotes restoration of acid-forming function of the stomach.
4. At endoscopic study there has been established rapid and adequate healing of ulcerous defects against the background of fullerene use, which claims recurrence-free effect of the therapy.
5. Indication of fullerene increases the effectiveness of eradication therapy due to increase of H. pylori susceptibility to antibacterial preparations.
6. Fullerene has significant antioxidative peculiarities, associated with full termination of negative free radical processes.
7. Normalization of protein exchange indexes in DU patients against the background of therapy with fullerene was conditioned by improvement of the processes of aminoacid absorption, functional state of hepatocytes, and inhibition of proteolytic “attack”.
8. Indication of BAS fullerene in the complex of conventional treatment of DU patients promoted normalization of cellular and humoral components of the immunity, which was associated with completion of the inflammatory process, improvement of the functional state of immune cells and restoration of barrier properties of the mucous coat of gastrointestinal tract.

PRACTICAL RECOMMENDATIONS

BAS fullerene, which is a water solution of hydrated C_{60} fullerene, can be recommended for use in complex therapy in duodenal ulcer patients according to the following regimen: 50 ml 15-20 min before meals 3 times a day for the first 3 days, twice a day – for the following 3 days, once a day – for the following 15 days.

For stabilization of the obtained results in this patient population it is advisable to resort to prolonged therapy with fullerene for 2 month – 50 ml once a day.

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