

Clinicoimmunologic aspects concerning use of new Ukrainian medicine Erbisol in combined therapy of patients with hepatitis

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Presented are data about the use of new Ukrainian-made medicinal product Erbisol in combined therapy of patients with chronic persisting hepatitis. Hepatoprotecting and immunomodulating properties of Erbisol are presented in detail, as well as original mechanism of its effect on pathological processes. Erbisol was shown to produce a more marked effect compared to traditional therapy. It was concluded that Erbisol is an effective and safe preparation for pathogenetic therapy of chronic persisting hepatitis and can be successfully used for treatment of hepatic disorders of varying genesis.

During the last decade an impetuous increase in morbidity of chronic hepatitis is noted but its treatment remains one of the difficult and outstanding problems⁵. Therefore the its search of new hepatoprotector agents able to increase an efficiency of the treatment in such category of patients and their introduction into therapeutic practice are highly actual².

In this respect Erbisol a representative of a new class of endogenic regenerative biological immunomodulating agents developed in Scientific Production Center "ERBIS" is the perspective drug. Erbisol is extracted from the animal embryonic tissues and contains a complex of the natural protein-free organic compounds of non-hormonal origin with low molecular weight⁶. The result obtained studying the mechanism of activation of regenerative processes in the animal organs and tissues were assumed in a basis of drug development. The author of a conception (confirmed by patents of the Ukraine N 2163 and N 2164; of Russia N2041715 and N2041717; international patents PCT/UA93/0003 and PCT/UA93/0003) Nikolayenko A.N. revealed that specific membrane glycoproteins ("markers of cellular physiologic state") determining the tissue immunogenicity and providing the process of information transmission of physiological state changes in the cells are located on the surface of a majority of the animal cells. Under normal cellular state, the "signalling" parts of markers for the body immune system are imperceptible but under pathological processes the conformation of their carbohydrate component and in accordance with this molecular immunogenicity are changed. The immune system is immediately reacted to these changes that are the alarm signal⁷.

Erbisol drug contains the "signalling" parts of "markers of cellular physiologic state" extracted from embryonic cells of animal tissues where the processes unusual for normal, standard state for adult organism take place. Administrating such "signalling" molecules into the organism "alarm signal" is introduced into the organism too and the immune system makes more active for a search of pathological focus.

The pharmacologic properties and activity of Erbisol are estimated by a content of its biologically active peptides in particular specific glycopeptides that activate the immune system for the search and elimination of pathological changes in the organs and tissues. The drug inhibits the processes of lipide peroxidation in

the membranes of animal hepatocytes during intoxication, increases an activity of blood glutathione dependent antioxidant system without changing an enzyme activity of microsomal oxidation and cytochrome p-450 content. Erbisol displays the antiinflammatory properties, normalizes the hepatocyte function, has membranestabilizing effect on the level of plasmatic membranes, prevents the development of dystrophy and cytolysis in hepatic disorders. Hepatoprotective, antiinflammatory and reparative properties of Erbisol are combined with its immunomodulating effect that is reflected as an increase in activity of macrophages (predetermining the reparative processes of damaged cells and restoration of organ and tissue functional activity), and also N- and T-killers responsible for the elimination of damaged or anomalous cells (mutant, malign, carrier cells and so on) and tissues not capable for the regeneration. Depending on the initial immune status of the body Erbisol modulates the activity of T-helpers, T-suppressors, B-lymphocytes and some other factors of humoral and cellular immunity¹², induces the synthesis of interferone and factor of tumor necrosis.

It is necessary to note an original mechanism of Erbisol action on pathological processes. Influencing not so much on disease as body's state and making more active the control systems of organism specifically the immune system, Erbisol initiates the search and elimination of pathological changes and also accelerates the restoration of damaged cells and elimination of anomalous cells and tissues⁷. It is only affected the pathological processes and is indifferent to the healthy organism. Normalizing the parameters of homeostasis the drug effect on the organism becomes minimum with correcting effect in accordance with a principle of back relation.

Erbisol is believed to intoxic substances without comulative properties and does not provoke allergic, cancerogenic, teratogenic and mutagenous effects. It is successfully used in treatment of erosive ulcerous damages of gastrointestinal tract^{8,10}, pyo-ceptic wounds and trophic ulcers in the vascular surgery¹¹, in therapeutical stomatology⁹, oncology^{3,4}, and endocrinology¹.

In accordance with the decision of Pharmacological Committee of the Ukraine (the extract from the protocol N 7 from 21.09.1995) Erbisol therapeutical effectiveness was studied in treatment of chronic diffuse diseases of the liver in Clinics of Hospital Therapy N2 at National Medical University.

Materials and methods

There were 85 patients with chronic persisting hepatitis (CPH) aged 17-63 years 58 men and 27 women among them who were examined by us. Data of clinical anamnesis were indicative of infectious or toxic damages were starting point of chronic diffuse disease of the liver. The duration of disease consisted of 2 to 8 years. An activity of inflammatory process in hepatitis corresponds to the minimal and moderate degrees. 65 patients of main group were received Erbisol combined with basic therapy excluding immunodepressants, immunomodulators, glucocorticoids, hepatoprotector agents every day intramuscularly in a dose of 2 ml for 20 days. Control group consisted of 20 patients who were under complex therapy without Erbisol treatment. The groups of patients were equivalent according to the clinical characteristics.

Complex clinical laboratory examinations including blood and urine counts and biochemical analysis (determination of total blood protein by biuret test and protein fractions by electrophoresis method on the paper by Gurvich, bilirubin number by Jendrashek, transaminase activity by Raitman and Frenkel methods, cholesterol content by Ilk, number of B-lipoproteids by turbidimetry method), ultrasonography analysis of the liver, the pancreas, bile passages, duodenal sound, roentgen exploration were realized in all the patients before and after treatment.

Immunological reactivity was evaluated by the dynamics of indices in cellular and humoral immunity, and by the parameters of unspecific protection. The relative and absolute number of the cells concerning basic subpopulations of T- and B-lymphocytes, natural killers (N-killers), monocytes, granulocytes was studied by the methods of laser flowing cytofluororhythmy with monoclonal antibodies against basic clusters of mononuclear differentiation in peripheral blood (CD₃, CD₄, CD₈, CD₁₄, CD₁₆, CD₁₉, CD₄₅, CD₅₆, HLADR); phagocyte activity of leucocytes (PAL), content of serum immunoglobulins A, M, G, circulating immune complexes (CIC), titres of antihepatic antibodies in blood serum were also investigated by us.

The manifestation of clinical syndromes such as dyspeptic, painful, fever, jaundice, astheno-vegetative, portal hypertension and hepatosplenomegaly were studied.

Results of clinical studies of Erbisol drug

Erbisol treatment exerted the marked positive effect that was expressed as the improvement of feeling and general condition, an increase in physical activity, the improvement of appetite, a disappearance or weakening of main clinical syndromes manifestation in patients with chronic persisting hepatitis.

Table 1.

MANIFESTATION OF CLINICAL SYNDROMES IN PATIENTS WITH CPH

Syndromes	Main group Erbisol n=65		Control group n=20	
	Before treatment, %	After treatment, %	Before treatment, %	After treatment, %
Astheno-vegetative	73,8	6,2	75	10
Painful	63,1	0	65	5
Dyspeptic	44,6	0	45	10
Jaundice	6,2	0	10	0
Hepatosplenomegaly	87,7	72,3	90	70

Data listed in Table 1 shown that the principal signs of exacerbation of hepatitis such as pain, dyspepsia, asthenia of the organism were succeeded with Erbisol treatment in the majority of patients by the end of treatment. Such treatment connected with an influence on the clinical manifestation of CPH is in accordance with traditional ones and exceeds it in some cases significantly.

In the most patients with CPH the changes in blood biochemical indices were noted before treatment (Table 2, 3). So, anaemia was revealed in 43-45% patients, the positive sedimentary tests (thymol test $37,2 \pm 1,1$ units) – in more than 1/3 patients and hyperbilirubinemia ($32,8 \pm 2,02$ $\mu\text{mol/l}$) – in a half of patients. An increase in the

Table 2.

DYNAMICS OF BLOOD CLINICO-BIOCHEMICAL INDICES IN PATIENTS UNDER INFLUENCE OF TRADITIONAL TREATMENT

INDICES n=20	Before treatment		After treatment			
	number	%	Normalization, %	Decrease, %	Without changes, %	Increase, %
Anemia	9	45	44,5	0	22,2	33,3
Increase in sedimentation test	8	40	50	12,5	25	12,5
Hyperbilirubinemia	10	50	50	20	20	10
Increased activity of AlAT	8	40	50	0	25	25
Increased AlkPh activity	6	30	50	0	16,7	33,3
Thymol test	7	35	57,1	14,3	14,3	14,3
Hyperproteinemia	8	40	50	12,5	37,5	0

Table 3.

DYNAMICS OF BLOOD CLINICO-BIOCHEMICAL INDICES IN PATIENTS UNDER INFLUENCE OF ERBISOL TREATMENT

INDICES n=65	Before treatment		After treatment			
	number	%	Normalization, %	Decrease, %	Without changes, %	Increase, %
Anemia	28	43,1	64,3	0	17,9	17,9
Increase in sedimentation test	25	38,5	60	30	10	0
Hyperbilirubinemia	32	49,2	59,4	28,1	12,5	0
Increased activity of AlAT	26	40	61,5	19,2	0	19,2
Increased AlkPh activity	20	30,8	65	15	10	10
Thymol test	24	36,9	80	20	0	0
Hyperproteinemia	8	40	50	12,5	37,5	0

activity of AlAt ($1,43 \pm 0,11$ $\mu\text{mol/l}$) and AcAt ($0,96 \pm 0,05$ $\mu\text{mol/l}$) was revealed in 40% patients. Dysproteinemia was found in 40% patients as a result of an increase in B- and G-globulin ($15,1 \pm 0,29\%$ and $23,8 \pm 0,13\%$, respectively). The mean data of

other biochemical indices did not exceed the limits of normal values before treatment and did not differ from those of control group.

Against a background of Erbisol use a significant ($P < 0,05$) decrease in the number of bilirubin, alkaline phosphatase, AlAt and AcAt and also in the level of thymol test was noted in the blood of patients (Table 3).

Content of total protein, globulin fractions of blood serum, A/G coefficient were normalized. According to the results of influence on these parameters, Erbisol treatment was the more effective in comparison with the results of traditional treatment.

Thus, Erbisol treatment has marked positive effect manifested in the improvement of feeling and general condition, an increase in physical activity, the improvement of appetite, a disappearance or weakening of manifestation of main clinical syndromes and also in the normalization of clinicobiochemical indices of the blood in patients with CPH.

Study of immune status in patients with CPH demonstrated (Table 4) that during the exacerbation the leucocyte and lymphocyte contents were within the limits of seasonal and age norms. However a significant ($P < 0,01$) decrease in percentage content and in absolute number of T-lymphocytes consisted of $49,30 \pm 11,15\%$ and $0,585 \pm 0,047 \cdot 10^9/l$ cells in ill patients while - $65,83 \pm 12,31\%$ and $0,970 \pm 0,038 \cdot 10^9/l$ lymphocytes - in healthy persons was revealed. In this case the content of T-helpers corresponded with lower limit of the norm and of T-suppressors exceed the limits of lower values of the norm. In 33% cases a significant decrease in the content of T-suppressor cells was noted and a correlation between helpers and suppressors was disturbed in these patients. A little increase in the number of B-lymphocytes (including an active ones) was detected in both groups of patients. The content of serum immunoglobulins in patients was as follows: IgM – $1,32 \pm 0,14$ g/l; IgG – $13,80 \pm 0,62$ g/l; IgA – $2,83 \pm 0,34$ g/l in patients of main group and IgM – $1,31 \pm 0,20$ g/l; IgG – $13,50 \pm 0,54$ g/l; IgA – $2,90 \pm 0,23$ g/l in patients of control group that corresponds with the limits of upper limits of the norm. However antihepatic antibodies were noted in 35% cases from 20 to 50 c.units in peripheral blood of patients. A significant increase in CIC content consisted of $101,7 \pm 10,4$ units was determined in patients of main and control groups.

Probably a decrease in a number of T-suppressors involves an increase in activity of B-lymphocytes resulting in the increase of immunoglobulin synthesis where an increased content of autoantibodies is noted among them and increased CIC content of patient blood in the end.

Disorders in the immune system also affect an unspecific function of the protection including polymorphonuclear neutrophiles (phagocyte index $74,1 \pm 8,1\%$, phagocyte number $7,9 \pm 0,4$, NST-test $79,5 \pm 8,1\%$; in norm $80,2 \pm 8,1$; $9,8 \pm 0,9$; $42,3 \pm 4,3\%$, respectively).

Table 4

Indices of the immune system status in patients with CPH after treatment course

Immunologic indices	Norm of healthy person, %	Patients with CPH		
		Before treatment %	After Erbisol treatment, %	After traditional treatment, %
CD45+14 Lymphocytes	15 – 35	26,43 ± 10,16	21,96 ± 4,83	25,14 ± 7,32
CD3+19 T- lymphocytes	45 – 75	49,30 ± 11,15	64,32 ± 6,84*	58,41 ± 8,46
CD3+DR T- active lymphocytes	5 – 10	6,10 ± 1,24	7,68 ± 3,67	7,54 ± 3,02
CD4+8+ Immature T- lymphocytes	0 – 1	1,06 ± 0,29	0,96 ± 0,22	1,01 ± 0,20
CD4 T- helpers	25 – 45	26,18 ± 7,95	36,37 ± 5,47*	30,69 ± 6,27*
CD8 T- suppressors	25 – 35	23,52 ± 4,27	29,82 ± 3,92*	25,14 ± 3,71
CD4/CD8 K=help./suppress.	1,2 – 2,3	1,11 ± 0,12	1,22 ± 0,02	1,22 ± 0,08
CD3+16+56 T- killers	3 – 8	3,46 ± 0,89	4,93 ± 0,98*	4,32 ± 1,01
CD3-16+56+ N-killers	7 – 13	5,85 ± 1,57	9,24 ± 2,25*	7,16 ± 1,97*
CD19 B-lymphocytes	6 – 12	10,24 ± 3,87	6,24 ± 2,03	8,42 ± 2,53
CD19DR B-active lymphocytes	6 – 12	13,56 ± 4,06	8,84 ± 3,64*	10,21 ± 4,37
CD14 Monocytes	3 – 8	5,14 ± 1,83	7,45 ± 2,23*	6,25 ± 2,18
CD45 Granulocytes	50 – 65	56,76 ± 11,48	65,12 ± 14,02	67,07 ± 12,63
CIC	20 – 55 c.u.	101,70 ± 10,43	67,54 ± 10,06*	82,14 ± 11,31*
Autoantibodies against hepatocytes	0 - 8 c.u.	32 ± 16	4 ± 4*	16 ± 8
IgA	1,5 – 3,0 g/l	2,83 ± 0,34 g/l	2,32 ± 0,32 g/l	2,37 ± 0,30 g/l
IgG	7,0 – 13,0 g/l	13,80 ± 0,62 g/l	9,54 ± 1,04 g/l*	10,21 ± 1,23 g/l*
IgM	0,7 – 1,3 g/l	1,32 ± 0,14 g/l	1,06 ± 0,23 g/l	1,19 ± 0,19 g/l

Note: * - significance before and after treatment $P < 0,05$

Therefore a presence of second immune insufficiency was established in patients with CPH who were under our observation. The latter is reflected by such changes of cellular and humoral immunity that contributes to the activation of namely autoimmune processes in the majority part of patients.

Erbisol use in the treatment of patients with CPH induced a significant ($P < 0,05$) increase in relative and absolute number of T-lymphocytes ($64,32 \pm 6,84\%$ and $0,873 \pm 0,056 \cdot 10^9/l$ cells). A significant increase ($P < 0,05$) in absolute and relative content of T-helpers as well as T-suppressors up to the normalization of parameters of its content was found. During the process of treatment the restoration of T-helper/T-suppressor disturbed correlation was noted in a half of patients that was reflected on the dynamics of changes in mean data. In $1/2$ patients the restoration of the level of Natural killers (N-killers) and their activity and in $2/3$ patients the level of T-killer were observed respectively.

After our Erbisol treatment the number of B-lymphocytes was decreased against an increase of T-suppressor level and first of all a content of immunoglobulins IgG was also normalized. The level of blood serum autoantibodies was sharply decreased up to 0-8 c.units in these patients. And an effectiveness of the drug was manifested according to the elimination of immune complexes. The normalization of metabolic activity in polymorphonuclear neutrophiles, the increase in their content were noted in accordance with NST-test. However no significant changes in the phagocyte index and phagocyte number were found.

It should be especially noted that during Erbisol use a significant increase in a content of monocytes, macrophage precursors is observed, an activation of the reparative processes is one of their functions. It can contribute to reduction of damaged cells and to activation of regenerative processes in affected organs and tissues of patients with CPH. It is possible that an efficiency of Erbisol use in the treatment of patients with CPH is explained by an increase in monocyte (macrophage) content and activity.

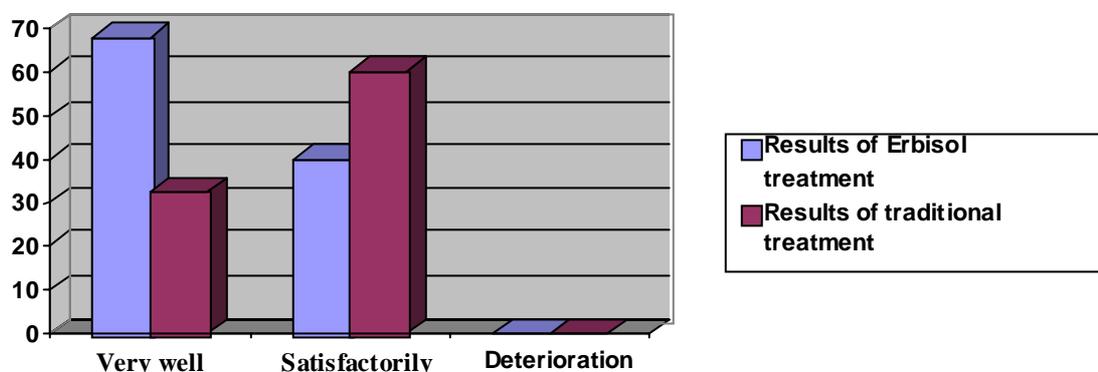
An improvement of the immune indices was less pronounced in the group of patients with CPH who were received traditional treatment. Specifically a content of T-suppressors was only increased up to the lower limits of a norm and a number of active B-lymphocytes remained increased as well as the level of autoantibodies and CIC in spite of normalization in the values of T-helper/T-suppressor coefficient. This demonstrates that the immunocorrection was defective in this case and autoimmune process was not stopped.

The carried investigations allow to conclude that Erbisol drug has the pronounced immunomodulating effect and influence on specific (T-helpers, T-suppressors, T-killers, B-lymphocytes) and unspecific (monocytes, granulocytes, N-killers) links of the immune system. Its effect is more directed at the normalization of the parameters of body immune status in this case and becomes apparent first of all under pathological processes. It should be noted that drug effect is also minimum under the minimum deviations in parameters of body immune status. Erbisol plays a role of immunocorrector and contributes to normalization of

disturbed immunological status by making more active T-lymphocytes. It being known that the drug increases a number of T-helper and T-killers and their functional activity, as well as of T-suppressors in a case of autoaggression. The latest inhibit the activity of B-lymphocytes that is important for stopping the autoimmune process (decrease in autoantibody titres and in CIC number) and for the restoration of a balance between cellular and humoral immunity. The restoration of a role of cellular immunity by the inhibition of B-lymphocytes in humoral immunity and the activation of N- and T-cellular immunity results in an increase of body's protective functions directed at the elimination of pathologic focus. T-killers eliminate the cell carriers contributing to the elimination of viral pathogene and N-killers eliminate yet more anomalous (regenerative hepatocytes) cells appeared for the first time. Thus, the cellular immunity plays the fixed role in the elimination of infection foci and inhibition of regenerative process of damaged cells into anomalous ones. Besides that Erbisol activates the cell and macrophage row for the reparation of damaged cells and the regeneration of ill tissue function.

Such an original immunomodulating effect of Erbisol was provided with the more marked effectiveness of complex therapy in patients with CPH side by side with indicated metabolic and antioxidant activity early (Fig.1).

Fig.1. Effectiveness of CPH treatment



In conclusion, the results of our experiments have been demonstrated that an arsenal of hepatoprotector agents was enriched with the new perspective and valuable preparation. Erbisol is effective and safe preparation for pathogenetic therapy of chronic persisting hepatitis and can be successfully used for treatment of varying genesis.

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