**The most studied and grounded fields of Tamerit application**

THE MOST STUDIED AND GROUNDED FIELDS OF TAMERIT APPLICATION

          ***Toxico-septic diseases and acute gastrointestinal infections***

          Scientific researches of pathogenesis of toxico-septic diseases and search of novel approaches to treatment of these conditions started in 1982 in laboratory for investigation of toxic and septic conditions (Department of Infectious Diseases of 1st Moscow Medical Institute /now it is called Sechenov Moscow Medical Academy/) under the leadership of professor M.T.Abidov. In this scientific field, 4 dissertations on competition for degree of doctor of medical sciences and 8 dissertations on competition for degree of candidate of medical sciences have been successfully completed and defended. Pre-clinical studies were carried out in different animal models of sepsis and toxic conditions using mice, rats, rabbits, pigs, cows. Several experiments were made using monkey and mink models. Experiments and clinical trials were performed in leading research and clinic institutes of former Soviet Union, as well as in Switzerland and Italy.

Septic and toxic conditions were modeling by intravenous or intraperitoneal injections of different doses of gram-positive and gram-negative microbes or endotoxin (lipopolysaccharide) of gram-negative bacteria (Salmonella, Shigella, Escherichia).

Clinical trials were performed in more than 3 thousand patients with food toxicoinfection, salmonellosis, shigellosis, other bacterial and viral infections causing enterocolitis or colitis.

Besides clinical symptoms of the diseases, in clinical trials and experiments we studied the following indices:

-         general blood and urine test,

-         cellular and humoral immunity,

-         concentrations of prostaglandins and cyclic nucleotides in tissues,

-         indices of clotting system,

-         adrenal function,

-         microcirculation,

-         content of alkaline phosphatase, myeloperoxidase, glycogen in blood cells,

-         phagocytic activity, spontaneous and stimulating chemoluminescence of neutrophils and macrophages.

Therapeutic activity of Tamerit was estimated in randomized trials, in which effects of Tamerit were compared to effects of placebo or well-known preparations widely used in treatment of above diseases (antibiotics, steroid and non-steroid anti-inflammatory drugs).

**Viral and toxic hepatitis**

Special attention have been paid to trials of Tamerit in models of toxic hepatititis as well as in patients with viral (A,B,C,D) and drug-dependant hepatitis (total number of patients – 600).

Besides estimation of clinical appearance of the diseases, in experiments and clinical trials and we studied the following indices:

-         general blood and urine test,

-         morphology of liver tissue,

-         antibody response to viral antigens,

-         detection of viral RNA or DNA (PCR) both in the blood and in the liver tissue,

-         level of liver enzymes and pigments in blood serum,

-         indices of lipid, protein and carbohydrate metabolism,

-         cellular and humoral immunity,

-         concentrations of prostaglandins and cyclic nucleotides in liver tissues,

-         content of alkaline phosphatase, myeloperoxidase, glycogen in blood cells,

-         phagocytic activity, spontaneous and stimulating chemoluminescence of neutrophils and macrophages.

Therapeutic activity of Tamerit was estimated in randomized trials, in which effects of Tamerit were compared to effects of placebo or well-known preparations widely used in treatment of hepatitis (ethiotropic medicines /interferon-α, ribavirin, lamivudin/ alone with standard detoxication and choleretic drugs).

Besides, we have studied the efficacy of Tamerit in patients with hepatitis associated with HIV infection (>150 patients) and lung tuberculosis (>200 patients). In these patients alone with above indices, we studied the following criteria:

*a)*    *hepatitis + tuberculosis*

-         x-ray criteria

-         bacteriological criteria (*M.tuberculosis*)

-         clinical (tuberculosis) criteria

*b)*    *hepatitis + HIV infection*

-         clinical and laboratory signs of HIV-associated diseases

-         qualitative and quantitative detection of HIV-RNA

***Erosive and ulcerative diseases of gastro-intestinal tract***

We have carried out 2 sets of clinical trials of Tamerit in patients with a) gastric and duodenal ulcers (N > 200), b) nonspecific ulcerative colitis (N > 120).

Both trials were performed as a randomized investigations, in which effects of Tamerit were compared to effects of well-known preparations widely used in treatment of gastro-duodenal or colonic ulcers.

We studied dynamics of clinical symptoms as well as the following indices:

-         general blood and urine test,

-         cellular and humoral immunity,

-         esophagogastroduodenoscopy or colonoscopy,

-         concentrations of prostaglandins and cyclic nucleotides in tissue samples from ulcers,

-         indices of lipid, protein and carbohydrate metabolism,

-         content of alkaline phosphatase, myeloperoxidase, glycogen in blood cells,

-         phagocytic activity, spontaneous and stimulating chemoluminescence of neutrophils and macrophages.

**Rheumatology**

We have studied the efficacy of Tamerit in MRL/l mouse model of autoimmune disease (systemic lupus erythematosus and rheumatoid arthritis)

          We estimated the following indices:

-         Mean life-span

-         Serum level of pro-inflammatory cytokines

We have data on values of IL-1 and TNF in MRL/lmice at 45 days of age, placebo-treated  and Tamerit-treated mice at 150 days of age.

The mice at 45 days of age usually have no sings of autoimmune disease and may be considered as intact and healthy. Therefor we use the values of serum concentrations of TNF and IL-1 on day 45 as control.

On this day, both TNF and IL-1 concentrations were less then level of detection (50 pg/ml).

On day 150, TNF concentrations in placebo-treated mice were equal to 215+66 pg/ml, IL-1 concentrations varied in larger degree and in many cases were lesser then level of detection (mean value = 140+95 pg/ml).

In Tamerit-treated mice (at a dose of 2 mg/kg twice a weak beginning from day 120) TNF concentrations in blood serum were equal to 112+47 pg/ml.

-         Pro-inflammatory cytokines in synovial liquid

-         Skin lesions

All mice in control group had skin lesions on day 150. In Tamerit-treated group, 50 (Tamerit at a dose of 2 mg/kg twice a weak beginning from day 120) or 80% (the preparation at a dose of 2 mg/kg twice a weak for 1 month beginning from day 45) of mice were fully free of skin lesions.

-         Morphology of kidneys, joints, lymphoid organs

-         Weight index of spleen

Besides, we performed clinical trial of Tamerit in patients with juvenile rheumatoid arthritis (RA) (N = 60). This trial was randomized, the efficacy of Tamerit was compared to preparations traditionally used in treatment of RA (non-steroid and steroid anti-inflammatory drugs).

Besides estimation of clinical symptoms of the diseases, we studied the following indices:

-         general blood and urine test,

-         rheumatoid factor and acute phase proteins in blood serum,

-         level of pro-inflammatory cytokines, prostaglandins and cyclic nucleotides in blood serum and synovial liquid,

-         detection of DNA (PCR) of viruses of herpetic group in the blood,

-         indices of lipid, protein and carbohydrate metabolism,

-         cellular and humoral immunity

Immunotropic Activity of Tamerit

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Elaboration of immunotropic preparations and me­thods for selective immunocorrection attracts much recent attention. Immune reactions underlie biologi­cal processes. Various components of the immune system are involved in the response to foreign agents and tissue damages, reparation, inhibition of tumor growth, elimination of transformed cells, and hemo-poiesis. Immunocompetent cells protect the organism from exogenous and endogenous pathogenic factors and play a key role in the pathogenesis of some dis­eases.

Pathogenetic studies of etiologically different infectious diseases revealed general principles of the development of inflammatory reactions. Macropha-ges first interact with infectious agents penetrating the mucosal and skin epithelium. The degree and type of body's reactions to foreign agents depend on the response of these cells.

Hyperactivation of monocytes and macrophages leads to the release of tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, NO compounds, prosta-glandins, and reactive radicals. These substances pro­duce para- and endocrine effects and cause local and systemic inflammatory reactions.

Inflammation is always accompanied by the in­volvement of monocytes and macrophages and inten­sive production of biologically active substances, which aggravates cellular and vascular disturbances. Therefore, pathogenetic therapy of inflammatory dis­eases (independently on their etiology) should in­clude reversible inhibition of hyperactivated mono­cytes and macrophages in the acute period.

The search for chemical compounds modulating functional activity of macrophages demonstrated that aminophthalhydrazides are most potent in regulating functions of monocytes and macrophages. We elabo­rated a new preparation Galavit, which possessesconsiderable antiinflammatory, immunomodulatory, and antioxidant properties.

Galavit causes reversible and short-term (6-8 h) inhibition, but not stable suppression of hyperacti-

vated macrophages. Moreover, Galavit normalizes cytokine production and antigen presentation in ini­tially suppressed monocytes and macrophages. Stu­dies of biological activity of Galavit indicate that this preparation modulates or regulates functions of ma­crophages, but does not suppress these cells. The effects of Galavit depend on its dose, course of treat­ment, and the initial state of target cells.

The efficiency of Galavit during the therapy of patients with infectious diseases is associated with its ability to activate the microbicidal system in neutro-philic granulocytes and phagocytosis of foreign agents.

Tamerit consists of 2 highly active aminophthal­hydrazides. The preparation was approved by the State Pharmaceutical Committee (registration certi­ficate No. 2000/113/5, 03.04.2000). Tamerit causes more pronounced and long-term biological effects than Galavit (10-12 h).

Similarly to other biologically active aminophthal­hydrazides, Tamerit possesses antiinflammatory, im­munomodulatory, and antioxidant properties. The major effects of this preparation are associated with its ability to modulate metabolism and functions ofmacrophages and neutrophilic granulocytes.

Antiinflammatory activity of Tamerit is related to reversible inhibition of production of TNF, IL-1, IL-6, NO compounds, reactive oxygen species, and other antiinflammatory factors by hyperactivated ma­crophages. The antioxidant effect of Tamerit is reali­zed through the decrease in oxygen consumption by hyperactivated macrophages and suppression of oxy­gen radical generation.

Immunomodulatory activity of Tamerit is mani­fested in normalization of antigen presentation and se­cretory functions of monocytes and macrophages, sti­mulation of the microbicidal system in neutrophilic granulocytes and cytotoxicity of natural killer (NK) cells, and immune-correcting effects in relation to cel­lular and humoral immunity. Tamerit enhances the bo­dy's resistance to infectious diseases and tumor growth.

Tamerit stimulates tissue reparation, activates the growth of granulations and epithelium, and acce­lerates epithelization of infected wounds and healing of ulcerative skin and mucosal lesions.

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The preparation stimulates synthesis of NO com­pounds during vascular disorders and decreases the vascular tone.

Moreover, Tamerit produces moderate bacterio-static effects on various infectious agents, including M. tuberculosis.

Combined treatment with easily soluble 5-ami-nophthalhydrazide sodium salt and hydrophobic 5-aminophthalhydrazide in the 1:1 and 1:0.5 ratios prolongs the therapeutic effect of this preparation.

Studies of general toxic properties of Tamerit in mice, rats, and dogs showed that the preparation is low toxic (class IV toxicity). Tamerit does not pro­duce toxic effects in animals during long-term ad­ministration in doses, which 10-15-fold surpass those used in clinical practice.

Before-clinical studies showed that Tamerit in therapeutic doses displays no mutagenicity in the Ames test and does not cause chromosomal aberra­tions in mammalian bone marrow cells, lethal domi­nant mutations in mouse germ cells, and DNA dam­ages. The preparation has no teratogenic, allerge-nic, and immunotoxic properties and does not affect reproduction and postnatal development of the off­spring.

Since antiinflammatory properties of Tamerit are associated with its effects on monocytes and macro-phages playing a key role in the immune system, we studied immunotropic activity of the preparation.

MATERIALS AND METHODS

Experiments were performed according to metho­dical recommendations on studies of immunotropic activity of preparations approved by the State Phar­maceutical Committee (record No. 10, 10.12.1998). We studied the effects of Tamerit on expression of some receptors on the surface of immunocompetent cells and peripheral blood neutrophil count in experi­mental animals. The ability of Tamerit to stimulate the antitumor immune response and enhance the effi­ciency of chemotherapy was evaluated.

The cells were cultured in RPMI-1640 medium (Flow Lab.) containing 5% inactivated fetal bovine serum (Flow Lab.), 2 mM L-glutamine, 5xl05 M 2-mercaptoethanol (Serva), 10 mM HEPES buffer (Flow Lab.), and 50 ug/ml gentamicin at 37°C and 5% C02.

In vitro experiments were performed on periphe­ral blood lymphocytes and segmented neutrophils from adult donors aging 25-40 years (n=5), spleno-cytes, and peritoneal lavage cells from BALB/c and C57B1/6 mice (Stolbovaya nursery).

In vivo effects of Tamerit in a dose of 2 mg/kg on leukocyte ratio were studied on 1-year-old Shin-shilla rabbits (Stolbovaya nursery).

Lymphocytes were routinely isolated from hepa-rinized peripheral blood of adult donors in a Ficoll-Verografin density gradient [12]. Interphase cells were washed 3 times with cold medium 199; their concentration was brought to 106 cells/ml. The count of viable cells was not less than 95%. Viable and died cells were studied by staining with acridine orange and ethidium bromide and then examined un­der a LUMAM-R-3 luminescence microscope [5]. Human Т lymphocytes were studied by the method described elsewhere [5]. Human В lymphocytes were estimated as cells carrying receptors to complement component III (EAC-RFC) [10]. Rosette-forming cells (RFC) were studied by staining with acridine orange and ethidium bromide and then examined under a luminescence microscope [5]. We synthesized most reagents.

The reaction of lymphocyte blast transformation (RBT) was performed by the method desribed else where [6]. Lymphoid ceils (5x105) were placed in a

96-well flat-bottom plate (Nunc) and incubated for 65 h. Concanavalin A in a dose of 2 µg/ml (Con A, Farmacia) and E. coli ОIII:B4 lipopolysaccharide in a dose of 100 ug/ml (LPS, Difco) served as nonspe­cific mitogens.

Cytotoxic activity of lymphocytes reflecting NK

cell activity was estimated using human erythroleukemia K562 cells at target cells [9]. The reaction of

neutrophilic granulocyte-mediated phagocytosis was

performed using latex particles [6]. Peripheral blood

cells were dried, fixed with methanol, and stained by

the method of Romanovsky-Giemsa [8]. Leukocyte

ratio was calculated routinely [4]. The results were

compared with published data [2].

The effects of Tament on the humoral and cellu­lar immune systems wee studied on 6-8-week-old CBA and С57Вl/6 mice (Stolbovaya nursery). The effect of this preparation on the formation of anti­bodies to sheep erytbrocytes was estimated by the method described elsewhere [7]. The mice received intraperitoneal injections of 107 erythrocytes in 0.5 ml physiological saline. Tamerit was injected intra­muscularly in single doses of 0.2, 2, 20, 200, and 2000 µg/mouse in 100 µl 0.9% NaCl simultaneously with sheep erythrocytes or 5 days after their admini­stration. Control animals were intramuscularly injec­ted with 100 ul 0.9% NaCl. The titer of antibodies (hemagglutinins) were measured 7, 14, and 21 days after immunization.

The effects of Tamerit on the cellular immune system were studied in the delayed-type hypersensi-tivity (DTH) reaction with sheep erythrocytes injec­ted subcutaneously in a dose of 106 cells in 0.5 ml 0.9% NaCl. Tamerit was administered intramuscular­ly in doses of 2, 20, and 200µg/mouse. On day 5,

108 sheep erythrocytes in 0.2 ml physiological saline were injected into the right hind limb pad. An equiva­lent volume of 0.9% NaCl was administered into the left hind limb. Local inflammation was analyzed 18 h after treatment. The right and left hind limbs wereweighted, and the reaction index was calculated.

Splenocytes and peptone-activated peritoneal macrophages from C57B1/6 mice served as IL-1- and TNF-producing cell [14]. The suspension of spleno-cytes or peptone-activated peritoneal cells (1 ml) was placed in a 24-well flat-bottom plate (Linbro). After

2-h incubation, nonadherent cells were removed by

3-fold washing with heated RPMI-1640 medium. The culture medium (2 ml) containing Tamerit in concen­trations of 0.1, 1, 10, and 100 ug/ml was added. The solution of Tamerit in the same concentrations (1 ml) was placed in wells immediately after addition of the cell suspension. Tamerit was dissolved in the culture medium immediately before the experiment. After 24-h incubation, supernatants were collected. The content of IL-1 was estimated using thymocytes from C57B1/6 mice as indicator cells [15]. TNF content was measured in the cytotoxic test with TNF-sen-sitive transformed L929 fibroblasts [11].

Tamerit in doses of 0.2, 2, 20, 200, and 2000 ug/ mouse was injected intraperitoneally to C57B1/6 mice 2 h before infection with  S.typhi and E. coli (LD100) to study the stimulation of nonspecific resistance. The ability of Tamerit to stimulate the antitumor im­mune response and potentiate the effects of cytostatics was studied on 2-4-month-old male C57B1/6 mice with melanoma В16 and Lewis carcinoma (Central Nursery of Experimental Animals, Department Kryukovo).

Melanoma В16 was grafted by subcutaneous in­jection of 2xl05 viable tumor cells into the hind limb pad. The therapy with Tamerit started 1 day after tumor inoculation. The preparation was injected intra­muscularly in a dose of 50 µg in 50 µl distilled water for 4 weeks (daily or 1 time per 3 days). Control animals received intramuscularly 50 µl distilled water. Tamerit-receiving and control mice were killed 28 days after treatment. We isolated the lungs and coun­ted macroscopically visualized metastases.

The suspension of 1.5-2.0xl06 Lewis carcinoma cells in 0.05 ml medium 199 was injected intramus­cularly into the shank. The volume of primary tumor focus and the number of metastases on the lung sur­face were estimated 21 days after inoculation. In thespecial series, we studied the effect of Tamerit on the average life span of mice with Lewis carcinoma.

Tamerit and cyclophosphamide were injected 2 times a week beginning from the 2nd day after ino­culation. These preparations were administered 5 times to study tumor growth and metastases. To eva­luate the average life span of animals, the prepara-

tions were injected 8 times. Cyclophosphamide was injected intraperitoneally in a dose of 25 mg/kg. Ta­merit was injected intramuscularly in doses of 50 or 500 µg/mouse. Control animals received an equiva­lent volume of 0.9% NaCl.

Con A-blasts were used as test systems to esti­mate the effects of Tamerit on IL-2 production. Sple­nocytes from BALB/c mice were stimulated with Tamerit for 40 h. IL-2 activity in the culture super­natant was measured. Splenocytes from C57B1/6 mice were stimulated with Con A for 3-4 days to obtain Con A-blasts. Culturing was performed in plastic flasks (Nunc) containing 5 ml cell suspension (5xl07 cells) and 2 µg/ml Con A. After incubation the mitogen was washed out from cells. The cells were placed in a 96-well plate (4xl04 cells/well). Test supernatants and 0.1 M a-methyl D-mannoside were added to inhibit the residual activity of Con A. The culture of Con A-blasts was incubated for 24 h. IL-2 content was estimated by proliferation of Con A-blasts (incorporation of labeled thymidine).

The results were analyzed by Student's t test.

RESULTS

Effects of Tamerit on Cellular Immunity

Tamerit stimulated expression of high-affinity re­ceptors to sheep erythrocytes on human lymphocytes. After preincubation of lymphocytes with Tamerit in doses of 1 and 10 ug/106 cells at 37°C for 1 h, the count of A-RFC increased to 51.4+4.0% (vs. 30±5% in the control, p<0.05). In a dose of 100 ug/106 cells Tamerit inhibited rosette formation (A-RFC count 8.4±1.5%, p<0.05) and blocked formation of "late" rosettes between human lymphocytes and sheep ery­throcytes reflecting the total number of human T lymphocytes. After preincubation of lymphocytes with Tamerit in a dose of 100 ug/106 cells, E-RFC count was 36.7±4.8% (vs. 60.5±6.4% in the control, p<0.05). Taking into account published data on the mechanisms of rosette formation between human lym­phocytes and sheep erythrocytes, it can be suggested that Tamerit in these doses modulates the adenylate cyclase system.

Tamerit in doses of 25, 50, and 100 ug/106 lym­phocytes increased the count of EAC-RFC (14.6±1.3

vs. 8.3±1.1% in the control, p<0.05). Tamerit in doses

of 1-10 ug/106 lymphocytes had no effect on receptor

expression. Coincubation of cells with Tamerit in

doses of 1-100 ug/105 lymphocytes for 16 h did not

change NK cell-mediated cytotoxicity. The index of

cytotoxicity was 38.3+5.4% (vs. 37.5±4.7% in the

control, p>0.05).​

 Tamerit in doses of 1-10 µg/105 lymphocytes sti­mulated spontaneous and LPS-induced proliferation of lymphoid cells (Table 1). In high concentrations (500 ng/105 cells) Tamerit blocked spontaneous and mitogen-stimulated cell proliferation. In all doses Tamerit suppressed Con A-induced cell proliferation (Table 1).

Stimulation of cell proliferation by Tamerit in low concentrations is probably associated with its ability to activate IL-2 production. In doses of 25 and 50 µg/ml Tamerit stimulated Con A-blast prolifera­tion (Table 1). Supernatants of splenocytes cultured with 50 µg/ml Tamerit displayed pronounced IL-2-like activity (Table 2). Tamerit was more potent than Con A in inducing IL-2 production.

The count of peripheral blood leukocytes (prima­rily segmented neutrophils) progressively decreased over the first hour after intravenous injection of 2 µg/ml Tamerit, but returned to normal 3 h later (Ta­ble 3). The percentage of lymphocytes did not differ from the control.

Tamerit in doses of 1-10 µg/107 segmented neu­trophils markedly increased the count of these cells. The degree of phagocytosis was 75% (vs. 55.5% in the control).

Preventive treatment with Tamerit in low doses (0.2 µg/mouse) 2-3-fold increased the average life span of animals intraperitoneally infected with E. coli and S. typhi in LD100.

Effects of Tamerit on Humoral Immunity

Effects of Tamerit on Humoral Immunity

The serum antibody liter in control mice increased 7 days after immunization with sheep erythrocytes, peaked on day 14, and decreased on day 21 (Table 4). On day 14, the antibody liter in highly reactive CBA mice 2-fold surpassed that in low reactive C57Bl/6 mice.

Seven days after treatment with Tamerit in the inductive phase of the immune response (simultane­ously with sheep erythrocytes), the hemagglulinin ti­ters in CBA and C57B1/6 mice did not differ from the control. Fourteen days after administration of Tamerit in doses of 0.2-200 µg /mouse, the concentration of serum antibodies in CBA mice was lowerthan in the control. However, Tamerit in doses of 20 and 200 µg /mouse increased the antibody titer in C57B1/6 mice. On day 21, the concentration of anti­bodies in highly reactive mice was above the control. During this period, the serum hemagglutinin liter in Tamerit-receiving mice did not differ from the control.

Treatment with Tamerit in the productive phase of the immune response (5 days after immunization)

suppressed antibody formation in CBA mice on days 7 and 14 after immunization (Table 4). However, Tamerit in doses of 2-2000 ug/ml stimulated hemag-glutinin production in C57B1/6 mice 21 days after immunization.

Tamerit in low doses increased the index of DTH reaction in all animals (Table 4). However, in high doses the preparation slightly inhibited this reaction in CBA mice.

Studies of IL-1 content in the culture supernatant of peptone-activate peritoneal macrophages coincu-bated with Tamerit revealed dose-dependent effects of this preparation on monokine production (Table 5). Tamerit in a concentration of 1 ug/ml markedly activated 1L-1 production (p<0.05). In a concentra­tion of 100 ug/ml Tamerit slightly decreased mono­kine production by peptone-activate macrophages.

Tamerit in a concentration of 10 ug/ml was most potent in stimulating IL-1 production by mouse sple-nocytes (p<0.05). In a concentration of 1 ug/ml Ta­merit only slightly activated this process (insignifi­cant). The preparation used in lower or higher con­centrations had no effect on IL-1 production.

We studied the effects of Tamerit on TNF pro­duction (Table 5). Tamerit stimulated cytokine pro­duction by splenocytes (1 and 10 ug/ml) and macro­phages (1 ug/ml, p<0.05). It should be emphasized that Tamerit in a concentration of 100 ug/ml inhi­bited TNF production by splenocytes (insignificant) and peritoneal macrophages (p<0.05).

Pharmacological properties of Tamerit are asso­ciated with its ability to modulate functional activity of monocytes and macrophages and production of some cytokines (e.g., IL-1 and TNF), NO compounds, and reactive oxygen species. Monocytes and macro­phages play an important role in the antitumor immu­ne response. Previous studies showed that carragee-nan-induced inhibition of these cells accelerates tumor growth and promotes metastases in animals, while sti­mulation of macrophages by immunomodulators sup­presses tumor cell growth and metastases. The data indicate a key role of monocyte and macrophages in the body's resistance to tumor growth. The adoptive trans­fer of activated macrophages and monocytes inhibits metastases from various tumors in animals and pro­duces positive therapeutic effects in patients with peri­toneal carcinomatosis (intraperitoneal injection).

Taking into account these data, we studied the effects of Tamerit on tumor growth, metastases, and life span of mice.

In most experiments Tamerit inhibited the growth of subcutaneous melanoma nodes and increased the life span of mice (Table 6, insignificant). Tamerit ad­ministered 1 time per 3 days retarded tumor growth (9%) and increased the life span of animals withmelanoma (15%). However, daily injections of this preparation produced less pronounced effects (5 and 6%, respectively).

We studied antimetastatic activity of Tamerit (Table 6). Tamerit injected in a dose of 50 µg 1 time per 3 days for 28 days significantly decreased the number of metastases into mouse lungs (by 44%, p<0.05). Daily injections of Tamerit produced lesspronounced effects (30%).

These antitumor properties of Tamerit are prob­ably related to stimulation of macrophages (the main target for biological activity of Tamerit). However,

the mechanisms underlying antimetastatic activity of Tamerit require further investigations.

 We studied the efficiency of Tamerit used alone (monotherapy) or in combination with cyclophos-phamide in mice with Lewis carcinoma. Tamerit de­creased the volume of Lewis carcinoma (Table 7). Tamerit in doses of 50 and 500 µg/mouse decreased the number of macroscopically visualized metastases in mouse lungs by 27% compared to untreated ani­mals. Tamerit in a dose 50µg/mouse potentiated the antimetastatic effect of cyclophosphamide. After combination therapy the number of metastases into the lungs decreased by 4 times compared to treat­ment with cyclophosphamide. Metastases were not found in 4 of 12 mice receiving combination ther­apy with cyclophosphamide and 50 µg/mouse Ta­merit.

Monotherapy with Tamerit had no effect on the average life span of mice with Lewis carcinoma (Ta­ble 7). Cyclophosphamide increased the life span of animals by 1 week. Tamerit did not potentiate the effect of cyclophosphamide on the average life span of mice (Table 7).

Thus, antitumor properties of Tamerit and its ability to enhance the efficiency of chemotherapy in mice with in vivografted tumors indicate that this preparation possesses primarily antimetastatic ac­tivity. The scheme of treatment with Tamerit during combination therapy of patients with malignant tu­mors should be optimized. Besides this, the mecha­nisms underlying antimetastatic activity of Tamerit require further investigations.

Our results show that Tamerit in doses of 1-10 µg/105 lymphocytes stimulates spontaneous and LPS-induced blast transformation of human lymphocytes. In higher concentrations (50-100 µg/105 cells) Tame­rit blocks spontaneous and mitogen-induced prolife­ration. Tamerit in doses of 1-10 µg/106 lymphocytes stimulates expression of high-affinity receptors to sheep erythrocytes on human Т lymphocytes. In high doses (100 µg/106 lymphocytes) Tamerit inhibits ex­pression of these receptors. Tamerit in doses of 25-100 µg/106 lymphocytes increases the count of cells carrying receptors to complement component III, but in doses of 1-10 µg/106 lymphocytes the preparation has no effect on expression of these receptors.

Tamerit in a dose of 2-3 mg/kg causes a transient decrease in the count of peripheral blood neutrophilic granulocytes, but does not change the percentage of lymphocytes. In a dose of 1-10 µg/106 cells Tamerit in vitro enhances phagocytic activity of neutrophilic granulocytes. Tamerit in doses of below 50 ug/ml stimulates IL-2 production.

Tamerit modulates antibody formation, which depends on its dose and initial immunoreactivity of the organism. Treatment with Tamerit in the pro­ductive phase of the immune response (5 days after administration of sheep erythrocytes) suppresses anti­body formation in highly sensitive CBA mice on days 7 and 14 after immunization. However, Tamerit con­siderably increases the hemagglutinin tilers in low sensitive C57B1/6 mice 21 days after treatment.

Tamerit in doses of 2 and 20 µg/ml activates the reaction of DTH in oppositely reacting mice. In a dose of 200 µg/ml Tamerit suppresses this reaction in CBA mice.

Tamerit in concentrations of 1-10 ug/ml mar­kedly activates production of IL-1 and TNF by peri­toneal macrophages and splenocytes. However, in a dose of 100 µg/ml the preparation inhibits production of these cytokines.

Tamerit in a dose of 0.2 ug/mouse increases the nonspecific resistance of mice to bacterial infections.

The ability of Tamerit to stimulate the antitumor immune response and enhance the efficiency of che­motherapy in mice with in vivo grafted tumors indi­cates that this preparation possesses antimetastatic activity. The preparation in therapeutic doses does not cause anaphylactic reactions.

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**Modulation of inflammatory response improves myocardial infarct healing in rats.**

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Abstract

It is reputed that the ideal therapeutic approaches to treatment of patients with acute coronary syndrome (ACS) and myocardium infarction (MI) should be aimed at the inflammation reaction triggers. This study investigated the effectiveness of the impact of L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines upon the course of experimental MI as compared to the impact of a preparation, officially registered in Russia as an immunomodulator, Tamerit, belonging to phthalhydrazid derivative substance. Acute MI in rats was induced by left coronary artery coagulation. Histological study of the myocardium sections and biochemical analysis has been carried out at the 1st and 7th days of the experimental MI. The conducted investigations have shown that under the action of immunocorrectors the inflammation reaction character changes, exudative/destructive inflammation is replaced by a proliferative-cellular one. Animals' blood biochemical analysis at the background of L-17 and Tamerit introduction has shown a decrease of aminotransferases and lactatedehydrogenases activity in blood as compared to the reference group of animals' indicators, which is evidently caused by epicardial injury of myocardium and lesser amount of the alternative cardiomyocytes. At the same time, no noticeable difference in biochemical characteristics in groups, having been treated to immunomodulators of different chemical composition was identified, which is the sign of the essential similarity of their impact. Thus, immunocorrectors of different chemical groups (Tamerit and compound L17) diminish the volume of initial myocardial infarction and accelerate the granulation processes in course of MI, and represent a new category of treatment agents.



Tetrahydrophthalazine Derivative »Sodium Nucleinate« Exert its Anti-Inflammatory Effects through Inhibition of Oxidative Burst in Human Monocytes

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ABSTRACT

We described the use of a new chemical substance Sodium nucleinate (SN) as an immunomodulatory substance exhibit- ing antiinflammatory properties. Sodium nucleinate (SN) registrated in Russian Federation as **Tamerit**®, is 2-amino- -1,2,3,4-tetrahydrophthalazine-1,4-dione sodium salt dihydrate, derivative of well known chemical substance luminol. To comprehend the mechanisms of SN immunomodulatory activity, we examined the SN modulation of the oxidative burst responses of whole blood human monocytes and polimorphonuclear cells (PMC) stimulated with phorbol 12-myri- state 13-acetate (PMA) or E. coli suspension in vitro. SN did not inhibit the proportion of neutrophils and monocytes phagocytosing E. coli. Oxidative burst responses of monocytes stimulated with PMA were strongly inhibited at SN con- centration ranging from 10–500 mg/ml, less efficient inhibitor was SN in E. coli stimulated monocytes (inhibition range was from 50–500 mg/ml SN). SN inhibited PMC oxidative burst only in range 100–500 mg/ml SN. In conclusion, we found SN as an efficient inhibitor of oxidative burst in monocytes. Since ROS generation in monocytes/macrophages has been found to be important for LPS-driven production of several proinflammatory cytokines, SN may exsert its anti- inflammatory effects through monocyte/macrophage oxidative burst inhibition.

Key words: oxidative burst, granulocytes, monocytes, Sodium nucleinate

Introduction

Neutrophils and monocytes/macrophages play an im- portant role in non-specific immune response and organ- ism resistance, specifically in anti-bacterial resistance and as effectors, inducing and regulating immune cells1. Free oxygen radicals kill bacteria in phagosomes and par- tially are released into the environment, intensifying killing microorganisms and simultaneously injuring the surrounding tissues. It is specifically intensified in acute inflammation, less in chronic course of the disease2,3.

Active oxygeninduced and free-radical-mediated oxi- dation of biological molecules, membranes, and tissues is closely related to a variety of inflammatory diseases. Once free radicals are produced, they multiply geometri- cally in chain reactions unless they are quenched by anti- oxidants or other free-radical scavengers. Antioxidants

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are compounds that react with free radicals and protect neighboring structures from oxidant damage. Common protective antioxidant nutrients include vitamins C, E, and P-carotene, however their action is limited due to their low capacity to enter tissue and cell compartments3,4.

This study was performed to determine whether So- dium Nucleinate (SN; 2-amino-1,2,3,4-tetrahydrophtha- lazine-1,4-dione sodium salt dihydrate, derivative of well known chemical substance luminol)) registrated in Rus- sian Federation as antiinflammatory drug Tamerit®, but in EU or USA not avaliable until recently, may play a role as inhibitor of respiratory burst in polymorphonuclear cells (PMCs) and/or monocytes. In previous study we demonstrated that SN is an efficient inhibitor of sepsis development in mice model of LPS-induced sepsis3. The

changes induced by SN included decreased mice plasma inflammatory cytokine production. Similarly we demon- strated a decreased TNF-a, IFN-g and IL-6 response in human LPS-stimulated PBMNCs.

Several authors have recently confirmed that ROS are important for normal lipopolysaccharide (LPS)-dri- ven production of several proinflammatory cytokines and for the enhanced responsiveness to LPS seen in cells from patients with chronic inflammatory diseases4,5. Mul- tiple cytokine secretion may be triggered by a vast num- ber of pathological conditions, many of them begin with activation of PMNs and/or monocytes4,5.

Since ROS was found to be important for lipopolysac- charide (LPS)-driven production of several proinflam- matory cytokines, we have focused on the in vitro influen- ce of the SN when applied in blood samples stimulating with agonists leading to an activation of respiratory burst in PMCs and/or monocytes.

Materials and Methods

Blood samples

Peripheral blood samples were drawn from 9 healthy individuals after obtaining their signed informed con- sents.

Phago-Burst Test

Phagocytosis and oxidative burst capacity was measu- red using Phago-Burst test (Orphegen Pharma, 341060+ 341058, Heidelberg, Germany). Whole blood test samples were divided into six plastic test tubes (BD, Falcon, 353052) in volume of 100 ml. All the tubes were held in ice bath during the preparation of the test. The test re- agents were added as follows: in the first tube FITC la- belled E. coli (phagocytosis control – FK); in the second FITC labelled E. coli (phagocytosis test – FT); in the third wash solution and 123 dihydrorhodamin (wash so- lution control); in the fourth unlabeled stimulating opso- nized E. coli and 123 dihydrorhodamin (E. coli stimu- lated test – EC); in the fifth fMLP and 123 dihydro- rhodamin (fMLP stimulated control – FMLP); and in the sixth PMA (phorbol myristate acetate) and 123 dihy-

drorhodamin (PMA stimulated test – PMA). Tube 1 was incubated at 0°C (FK) and all others on 37°C for 10 min- utes. Whole blood test samples were then lysed with lysing solution and washed three times with wash solu- tion. Cells were than stained with propidium iodide. Measuring was performed using flow cytometer (BD, FACSort, USA) equipped with argon-ion laser of 488 nm excitation wavelength.

FITC labeled E. coli suspension

FITC labeled E. coli (Phago-Burst test (Orphegen Pharma, Heidelberg, Germany) were used for the stimu- lation of monocytes/PMC. E. coli suspension (5 ́107/ml) was quantitated by using Cell Viability Kit with Liquid Counting Beads (BD Biosciences). The kit contains a liq- uid suspension of fluorescent beads, which are added to the flow sample to calculate absolute counts of bacterial suspension.

Statistical analysis

Descriptive statistics were calculated for all the mea- sured parameters. Differences between control and SN pre-treated samples were tested both using paired t-test and exact Wilcoxon signed-rank matched-pairs test (EWMPT). Differences were taken as statistically signifi- cant if p<0.05. Statistical analyses were performed using SPSS for Windows 15.0.1.1 software (SPSS Inc., Chicago, IL, 2007).

Results

Phagocytic activity of monocytes/neutrophils

The ability of neutrophils to phagocytize opsonized E. coli was assessed. No inhibitory effects of SN (range 0.1–500 mg/ml) were found in monocytes/PMC stimulat- ing either with PMA or E. coliin vitro (data not shown).

Inhibitory effects of SN on monocyte/neutrophil oxidative burst

To determine the effect of SN on oxidative burst of monocytes/MNCs blood was preincubated with 0 (con- trol), 0.1, 10, 50, 100 and 500 mg/ml of SN for 10 min.

Fig. 1. Dose effect for the inhibition induced by SN on the monocyte oxidative burst in PMA and E. coli stimulated blood. Data are pre- sented as X±SD (N=9). SN was added to whole blood 10 min before phago-burst test. Monocyte oxidative burst activity of normal blood (Neg control,) and blood containing SN in concentrations 0.1; 1; 10; 50 and 500 mg/ml). \* p<0.05; \*\* p<0.01.

Fig. 2. Dose effect for the inhibition induced by SN on the PMC oxidative burst in PMA and E. coli stimulated blood. Data are presented as X±SD (N=9). SN was added to whole blood 10 min before phago-burst test. PMC oxidative burst activity of normal blood (Neg con- trol,) and blood containing SN in concentrations 0.1; 1; 10; 50 and 500 mg/ml). \* p<0.05; \*\* p<0.01.

Blood was then stimulated with PMA or E. coli suspen- sion (5 ́107/ml). We assessed the effects of SN on mono- cyte/PMC oxidative burst activity as measured by the conversion of DHR 123 to rhodamine 123. When com- pared to PMA-activated monocytes which were cultured alone, monocytes preincubated with SN showed a signifi- cant decrease in oxidative burst activity (Figure 1). Oxi- dative burst responses of monocytes stimulated with PMA were strongly inhibited at SN concentration rang- ing from 10–500 mg/ml, however SN was less efficient in- hibitor in E. colistimulated monocytes (inhibition range was from 50–500 mg/ml SN). SN inhibited PMC oxida- tive burst only in very high concentration range (100 and 500 mg/ml SN).

Discussion

The oxidative burst assay employed in this study uti- lizes dichlorofluorescein diacetate as a fluorescent probe. This assay detects mainly superoxide anion and hydro- gen peroxide, and to a lesser extent, other reactive oxy- gen species such as hydroxyl radicals4. The inhibition of oxidative burst by SN would suggest its ability to scav- enge superoxide anion and hydrogen peroxide.

In the previous study, we first demonstrated that SN is an efficient inhibitor of sepsis development in mice model of LPS-induced sepsis. The changes induced by SN include decreased mice plasma inflammatory cyto- kine production. Similarly we demonstrated a decreased TNF-a, IFN-g and IL-6 response in human LPS-stimu- lated PBMNCs. SN was therefore shown to be a promis- ing inhibitor of multiple inflammatory cytokine secretion.

Inflammatory mediators, cytokines (e.g. TNF-a) in- crease the ability of monocytes/PMC to localize at the site of inflammation. Phagocytic ability is elevated by in- tensification of hydroxylic radical production and lyso- somal enzyme release (6). TNFa is an important factor strengthening granulocyte phagocytic and cytotoxic ac- tivity. Activated monocytes and granulocytes secrete cy- tokines. IL-1, which stimulates monocytes, endothelial cells, and fibroblasts to secrete IL-8, in turn increases the expression of CD11b/CD18 adhesive molecules and gra- nulocyte oxygen metabolism7,8.

This study was performed to determine whether SN may exert the anti-inflammatory effects by acting as as inhibitor of respiratory burst in PMCs and/or monocytes. Stimulation of monocytes/PMCs by phorbol 12-myristate 13-acetate (PMA) results in induction of superoxide an- ion production through assembly of the NADPH-oxidase complex. This so-called respiratory burst is fundamen- tally important in monocytes/PMC since it is implicated in the killing of microbial intruders and in the tissue damage secondary to the induced inflammatory respon- se9. The activation status of monocytes/PMCs can range from quiescent over primed to fully activated10.

To determine the effect of SN on oxidative burst of monocytes/MNC blood was preincubated with 0 (Control), 0.1, 10, 50, 100 and 500 mg/ml of SN for 10 min. We as- sessed the effects of SN on monocyte/PMC oxidative burst activity as measured by the conversion of DHR 123 to rhodamine 123. Blood was incubated without or with SN and after 10 min whole blood was stimulated with PMA or E. coli suspension (5 ́107/ml). When compared to mono- cytes which were cultured alone, PMA-activated mono- cytes showed a significant decrease in oxidative burst ac- tivity. Oxidative burst responses of monocytes stimulated with PMA were strongly inhibited at SN concentration ranging from 10–500 mg/ml. SN was less efficient inhibi- tor in E. coli stimulated monocytes (inhibition range was from 50–500 mg/ml SN). On contrary SN inhibited PMC oxidative burst only in range 100–500 mg/ml SN, that con- centration of SN is probably a lot over the achievable range during therapy and inhibitory effects may be a con- sequence of some relatively unspecific process.

We found SN as an efficient inhibitor of oxidative burst in monocytes. The data from this study are encour- aging as researchers across the world are searching for ways to prevent the development of free-radical-medi- ated disorders such as atherosclerosis, arthritis, cancer, and aging11,12. Furthermore our data may also explain in- triguing clinical data described in Russian pharmacopeia where SN is declared as a potent anti-inflammatory drug with excellent body-distribution and minimal side ef- fects3. As we found SN effective mainly as a potent ROS scavenger in strongly activated monocytes (by PMA as strong ROS activator), SN may exsert its antiinflamma- tory effects through oxidative burst inhibition in strongly activated monocytes/macrophages13. Since ROS gen- eration in monocytes/macrophages has been found by many authors to be important for LPS-driven production of several proinflammatory cytokines, the antiinflama- tory action of SN may be a consequence of ROS inhibi- tion in activated monocytes/macrophages2,13,14. Although SN used as a drug is widely distributed within tissues and cells through the body, its ROS scavenger function is phisiologically important mainly in cells where ROS pro- daction reaches substantial levels, e.g. in monocytes/ma- crophages and neutrophils.

Side effects of SN may be therefore expected in inhibi- tion od neutrophil killing of bacteria, an essential process

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in defence against infectious agents. However, we as- sesed the ability of neutrophils to phagocytize opsonized E. coli was assessed. No inhibitory effects of SN (range 0,1–500 mg/ml) were found in monocytes/PMC stimulat- ing either with PMA or E. coli in vitro. On the other hand, SN inhibited PMC oxidative burst only in range 100–500 mg/ml SN, that concentration of SN is probably a lot over the achievable range during therapy. It is therefore probable that SN does not affect neutrophil killing of bacteria while it is capable to act as ROS scav- enger and anti-inflammatory agent in monocytes/mac- rophages3,4.

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TETRAHIDROFTALAZINSKI DERIVAT »NATRIJ NUKLEINAT« ISPOLJAVA PROTUUPALNO DJELOVANJE KROZ INHIBICIJU OKSIDATIVNE AKTIVNOSTI HUMANIH MONOCITA

SA@ETAK

Opisali smo kori{tenje nove kemijske tvari Natrij nukleinata (SN) kao imunomodulatorne tvari koja pokazuje protu- upalna svojstva. Natrij nucleinat (SN) registriran u Ruskoj Federaciji kao Tamerit® je 2-amino-1,2,3,4-tetrahidroftala- zin-1,4-dion dihidrat natrijeve soli, derivat poznate kemijske tvari luminol. Da bi shvatiti mehanizme SN imunomodu- latorne aktivnosti, ispitali smo SN modulaciju naglog oksidativnog odgovora monocita i polimorfonuklearnih stanica (PMC) cijele ljudske krvi stimuliranih s forbol-12-miristat-13-acetatom (PMA) ili E. coli suspenzijom in vitro. SN nije sprije~io udio neutrofila i monocita u fagocitiranju E. coli. Nagli oksidativni odgovori monocita stimuliranih s PMA sna`no su inhibirani sa SN u rasponu koncentracija od 10–500 mg/ml, manje u~inkoviti inhibitor je SN u monocita stimuliranih s E. Coli (inhibicijski raspon od 50–500 mg/ml SN). SN je inhibirao PMC oksidativni odgovor samo u rasponu od 100–500 mg/ml. Kao zaklju~ak, otkrili smo da je SN u~inkoviti inhibitor naglog oksidativnog odgovora u monocita. Od kako se pokazalo da je produkcija ROS u monocitima/makrofaga va`na za LPS-potaknutu proizvodnju nekoliko proupalnih citokina, SN mo`e vr{iti svoje protuupalno djelovanje kroz inhibiciju monocitnog/makrofagnog naglog oksidativnog odgovora.

Abbrevations

LPS – lipopolysaccharide
ROS – reactive oxigen species
PBMNC – peripherial blood mononuclear cell

PMC – polymorphomononuclear cell TNF-a – tumor necrosis factor alpha IFN-g – gamma interferon
IL-6 – interleukin 6

### The Role of the System of Phagocyting Mononuclear in Regeneration of Various Tissues

The Role of the System of Phagocyting  Mononuclear in Regeneration of Various Tissues

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**Summary**. The functional state of the system of phagocyting mononuclear plays an important role in regulation of tissues regeneration. The activation of microphages results in acceleration of processes of regeneration regardless of the type of the injured tissue. The purposeful effect on the system of phagocyting mononuclear may be used for treatment for various diseases connected with regeneration of tissues injured by the pathological process of tissues.

**Key words**: the system of phagocyting mononuclears, macrophages, regeneration, tamerit.

Acknowledgment of the immune system (IS) to be of equal value with the nervous and endocrine regulating systems providing homeostasisof the organism inevitably raises the problem of decoding and working out in detail of regulating mechanisms of non-immunological functions of the organism by the immune system. In practice the effect of the immune system (IS) on the processes of  regeneration growth of tissues is of significant interest as the majority of extreme effects damage organs and tissues, and adapting and compensatory mechanisms depend on repair processes. The morphogenetic function of lymphocytes [2, 3] has been researched in detail to the greatest degree. There is a voluminous factual material which confirms the ability of lymphocytes to regulate the proliferation of somatic cells: endothelial, epithelial, smooth**-**muscle**,**hepatocytes**,**osteoblasts**,**etc. [3, 12, 14, 15]. The research of mechanisms of lymphoid regulation of regeneration of the blood-formingtissue has been described in a number of works [4, 5, 6].

At the same time participation of the system of phagocyting mononuclears (SPM) in regulation of the regenaration process of various tissues has not been studied, though there are a few researches carried out in vitro which demonstrate a likely role of phagocytes in regeneration of injured tissues [9, 17, 19]. It is well known that organs and tissues are characterized by their own specific peculiarities of regeneratingprocesses**.**There are slowly**-**and strongly**-**renewing tissues. However the problem of connection between the morphogenetic function of microphages and the type of regeneration of tissue has not been studied yet. At the same time the acknowledgement and evidence of participation of SPM in regulation of regeneration gives an opportunity of substantiating theoretically and working out methods of modulation of regeneratingprocesses based on the purposeful influence on microphages.

The object of this work is the research of the role of the system of phagocyting mononuclears in regeneration of various tissues.

# Materials and Methods of the Research

The experiments were carried out on white mongrel male mice of 180-200 g. weight kept on the ordinary ration of vivarium. The regeneratingprocesses were studied in the marrow and liver which have different types of regenerating processes, and also in the parodont which combines a complex of tissues with different regenerating reactions. Regeneration of the microcirculatory course of the muscle tissue was studied as well.

The repairregeneration of the lever was induced by removal of 2/3 of the mass of the organ. [6]. The research was carried out in 4 and 17 hours after the operation. It corresponded with the destructive-reactive and proliferation phase of the lever regeneration [11]. The level of the regeneratingprocesses was determined by measurement of the mass of the regenerating lever and the percentage content of the dry substance in the tissue as well; the nucleus- cytoplasmatic andmitoticindex, the number of double-nucleus cells per 1000 hepatocytes were calculated **on**(with the help of) histological preparations. The material for the histological research of the resected lever was taken from three parts of it: the first - directly near the place of the resection, the second - 1-2 cmfrom the place of the resection, the third - farther than 2 cm.

The regenerating processes in the marrow were researched on the model of the acute massive haemorrhage. The rats were anemised by taking blood from the caudalvein in size 2% of the body mass. The assessment of regeneration of theblood-forming tissue was carried out according to the indices of the peripheral blood and marrow. In the marrow the total number of mielocariocyteswas calculated, the calculation of the mielogram of the absolute number of erythroblastislets (**EI**) was made, and their typing according to maturity classes was carried out [7].

The domestic preparation “Tamerit”, the modulatorof activity of microphages, was injected intramuscularly an hour before the operation, the dose - 2 mg/ kg [10].

Ischemia of a hind limb was simulated by tying the femoral artery of the rats. Regeneration of microcirculation was carried out by a single intramuscularinjection of “Tamerit” ten days after the operation. The counter laterallimb with the tied artery in which the physiological solution was injected served as the control. 12 and 19 days after the injection the animals were taken off the experiment underether narcosis. The calculation of the number of the capillaries in the muscle was made in twenty random visual fields**,**area of each - 0,017 mm2**,**with the subsequent recalculation for 1mm2**.**

The model of periodontosiswas carried out according to the method [8]. Under the inhalation narcosis tooth-gumjointoflower front-teethwas destroyed with the subsequent administrationof the crumbled scale. Two weeks after the operation there were parodont-likechanges. Visually there were: dropsical gums, their hyperemia, pathological pockets pocketsin the area of the lower front-teeth, pus and mobility of the teeth.

On the fourteenth of the experiment “Tamerit” was injected into the gum, dose 2 mg/kg. The morphological changes were revealed on the histologicalsections of the paradont tissues.

The nontreated animals were the control set..

The computing of the data was made on the basis of the methods of variation statistics with the use of parametriccriteria, with the software package Microsoft Exel 7.0. The comparison of the average indices was made by the criterion of Student. In some cases the non-parametriccriteria of Mann - Witny was used.

# Results and Discussion

In blood-formingtissue which is a representative of  strongly renewing tissues with the prevailing regeneration of the cell type, the activation of microphages of anemisedrats increases the general cellularityof the marrow in both studied periods without any influence on the character and focus of changes of the indices of the peripheral blood in response to blood loss. The research of mielogramsshows that the effect of tamerit results in activation of all the shoots of the marrow: erythroid neutrophilic, monocytic and lymphoid**.** The number of monocytes -macrophages of the marrow increases sharply.

In the marrow the absolute content of erythroblast islets does not increase in comparison with the nontreatedanimals which were not given the preparation. However in the peripheral blood the increase of the amount of blood reticulocytesis observed during twenty four hours after blood loss, and it indicates the stimulation of erythropoiesis against the background of activation of macrophage activities (Table 1).

Table 1 - Indices of peripheral blood, mielogram, the absolute amount of erythroblast isletsof the rats’ marrow after blood loss against the background of activation of macrophages

|  |  |  |
| --- | --- | --- |
| Index | Intact | Blood loss |
| 1 day | 1 day+tamerit | 3 days | 3 days+tamerit |
| Absolute numberEIx 103/thigh | 118,89±11,51 | 128,24±8,90 | 100,28±15,75 | 235,06±15,75\* |  205,64±11,6\* |
| General cellularity of marrow mln/100g | 35,69±2,78 |  25,24±3,28\* |  58,31±4,89\*, \*\* | 34,68±3,77 |  55,71±5,61\*, \*\* |
| Erythriod row mln/100g | 0,98±0,04 |  2,37±0,16\* |  4,45±0,79\*,\*\* |  1,83±0,15\* |  5,87±0,69\*, \*\* |
| Neutrophilic row mln/100g | 7,13±0,64 |  1,46±0,09\* | 8,57±0,87\*\* |  4,64±0,31\* |  11,88±1,02\*, \*\* |
| Monocytic row mln/100g | 21,74±0,76 |  9,09±1,16\* |  30,69±2,78\*, \*\* |  16,29±1,13\* |  24,82±2,31\* ,\*\* |
| Lymphoid row mln/100g | 0,71±0,03 |  0,42±0,06\* |  1,09±0,08\*, \*\* | 1,31±0,39 |  2,91±0,26\*, \*\* |
| Blood cells, T/l | 3,91±1,22 |  8,89±1,01\* |  14,26±1,17\*, \*\* |  8,01±1,30\* |  13,93±1,25\* \*\* |
| Reticolocytes, G/l | 6,85±0,16 |  5,26±0,13\* | 6,60±0,29 |  5,64±0,23\* |  5,48±0,54\* |
| Leucocytes, G./l | 146,84±21,50 |  473,67±37,68\* |  837,76±92,07\*, \*\* |  717,18±60,00\* |  810,29±94,46\* |
| Monocytes, G/l | 11,45±1,14 |  14,77±1,16\* |  15,92±1,84\* |  15,34±1,06\* |  13,38±2,67\*\* |

\* P 0,05 relative to the intact animals

\*\* P0,05 relative to the nontreated animals

Thereforeit can be stated that activation of macrophages  promotes regeneration of the hemopoietic tissue after the massive bloodlossstimulating erythropoiesisand intensifying hyperplasia of those shoots of the marrow which are divided by mitosis (erythroid, granulocyte, lymphoid).

In the slowly renewing tissue with the marked intracell type of regeneration (liver) the stimulation of the macrophage activity of resected animals also results in intensification of regenerating processes in the gland. The mass of the surviving after the resection lever fragment increases in 4 hours after the operation in contrast to the control set, and in 17 hours the mass of the regenerating remainder grows much more strongly than of the nontreated rats. (Table 2).

Table 2 - The mass of the regenerating remainder of the lever, (M+m).

|  |  |  |  |
| --- | --- | --- | --- |
|   | 0 hours of regeneration | 4 hours | 17 hours |
| mg | % of increase | mg | % of increase |
| Control without preparation | 3746,91157,58 | 2850,0142,35 | 76,06 3,51 | 5232,50487,4 | 139,649,80 |
| Tamerit | 2698,00260,30 |  4026,0377,128\*,\*\* |  149,2213,98\* |  4931,6689,27\*\* |  183,565,27\* |

\* P0,05 relative to the index of the control set.

\*\* P0,05 relative to the index immediately after the operation.

During the histological analysis of the slices of the regenerating lever in three different zones it was discovered that under the influence of tamerit the number of mitoses increases at the early terms evenly in all the zones, and in 17 hoursto the utmost in the middle zone, which is apparently connected with preservation of the integrity of the cells and vascular race. At the same period the increase of the number of two-nucleus cells is observed. It agrees entirely with the research carried out before [2] which showed in the culture of the cells of various organs that macrophages stimulated by tamerit forwards the entry of the cells into S-phase of the mitotic cycle (Table 3).

The experiments with the resection of lever testify the fact that activation of microphages influences the speed and manifestation of the regenerating process, it stimulates the processes of  the intracellular and cellular regeneration both in the destructive-reactive and proliferation phases of the gland regeneration.

Table 3 - The mitotic index in the regenerating remainder, **‰,**(M+m).

|  |  |  |  |
| --- | --- | --- | --- |
|   | Area of lever | 4 hours after resection | 17 hours after resection |
| Control without preparation | Focus of resection | 0,25±0,25 | 0,25±0,25 |
| Middle | 0,25±0,25 | 0,25±0,25 |
| Remote from resection | 0 | 0,25±0,25 |
| Tamerit | Focus of resection | 1,0±0,77 | 2,0±0,71\* |
| Middle | 2,0±0,45\* | 5,5±0,13\*,\*\* |
| Remote from resection | 2,6±0,93\* | 2,8±0,73\*,\*\* |

\*P0,05 relative to the index of the control set

\*\*P0,05 relative to the index immediately after the operation

Activation of microphages forwards regeneration of the microcirculatory race in the ischemia limb of the femoral artery of the rats. The histological research shows that the experimental animals which got tamerit, both 12 and 29 days after the injection of the preparation have significant increase of the number of capillaries in comparison with the ischemia muscle injected with physiological solution.

At the same time the number of capillaries in the zone of ischemia 12 days after the injection of tamerit mounts to the values if the intact animals, and in 29 days the density of the capillaries is nearly 1,7 times higher (Table 4).

Table 4 - The formation of capillaries in the ischemia muscle during the injection of tamerit (per 1mm2)

|  |  |  |  |
| --- | --- | --- | --- |
| Indices | Intact | Ischemia 22 days | Ischemia 30 days |
| Physiological solution | Tamerit | Physiological solution | Tamerit |
| Sum of capillaries | 138,8±12 | 29,8±3 | 152,3±12,7\*\* | 31±3,2 |  236,4±16,9\*,\*\* |
| Blood cells (Leicocytes) | 5,3±2,2 | 2,2±0,7 | 0 | 0 | 0 |

\*P0,05 relative to the index of the intact animals

\*\*P0,05 relative to the index of the control set

The complexity and irregularity of regenerating processes of  parodont reesult from the variety of the composing tissues: gums, tooth-gum combination, periodont, interdental wall. On the histological preparations of the nontreated animals, the resorption of the bone tissue and focal destruction of the factorial islet and destruction of the ligamentary organ have been found. In the structures of parodont a manifestedinflammatory reaction accompanied by the lymphocyte infiltration with the impurity of granulocytes was observed. On the 14th day of the experiment suppurative inflammation persisted in the tissues of the periodont (Figure 1).

Figure 1 - Periodont of the nontreated rat at the 14th day after the experiment. The area of the resorption of the bone with evident lympholeucocyte infiltration of the adjacent tissues. The colouring with hematoxylin and eosin. Enlargement x200.

In the group of the animals given tameritcircularperivascular infiltrations are found in single instances only**.**The vessels of the microcirculatory race are full-blooded. The entire regeneration of the ligamentary organ of the tooth and decrease of the resorption of the bone tissue took place(Fig. 2). The factorial islets in the spongy bone of the bone  alveole with the signs of proliferation (Fig. 3), and - in half of cases - in the periodontal tissue and interadjacent substance of the legimentary organ there were aggregations of macrophages and fibroblasts with the signs of activity (Fig. 4).

Figure 2 - The legimentary organ **tooth-gum joint** of the rats treated with tamerit without signs of destruction. Colouringaccording to Van-Hison. Enlargement x 150

Figure 3 - The factorial islets with the signs of proliferation in the spongy bone of the bone  alveole of the rats treated with tamerit. Colouring with hematoxylin and eosin. Enlargement x 200

Figure 4 - The periodontal tissue and ligamentary organ of the rats treated with tamerit. Aggregation of microphages and fibroblasts with the signs of proliferation. Colouring with hematoxylin and eosin. Enlargement x 200

On the basis of the present researches it can be concluded that the system of phagocyting mononuclears is not only one of the inductors actuating the regeneration but it also regulates the speed and evidenceof the regenerating growth of tissues chatacterized by their peculiarities of regeneration processes. The mobilization of the activity of macrophages results in acceleration of regeneration processes irrespective of the type of the injured tissue. In the hematopoietic tissue erythropoiesis is stimulated which is confirmed by the development of reticulocytosis and hyperplasia of the erythroid shoot; in the liver the processes of the intracellular and cellular regeneration become stronger, in the ischemia musclethe regeneration of the microcirculatory race takes place, and in parodonttissues a faster regeneration of the structures of the periodontal tissue is marked.

The acknowledgement of the role of the macrophage link in the regulation of  regeneration allows to use the purposeful influence on the system of phagocyting mononuclears for treatment foe various diseases and their aftermaths connected with regeneration of tissues destroyed by a pathological process.

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**New Approaches in Peptic Ulcer Treatment**

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**New Approaches in Peptic Ulcer Treatment**

Intestinal infections and ulcer diseases are the most common gastrointestinal disorders. Nevertheless, despite the fact that the whole intestine, the area of which equals to 300m2 is settled with microorganisms (there are more than 100 billion microbes inside of a human body; the quantity of microbes on skin surface is 10 times higher than the amount of cells in the body), they are very seldom the cause of a disorder themselves. Micro- and macro organism symbiosis has been achieved through thousands of years and is determined both evolutionally and functionally. From the other side, more than a milliard microbes enter the organism daily, but the diseases appear very rarely on the reason that microbes’ antagonism works for the sake of macro organism.  However, constant usage of chemical drugs changes the balance for the microorganisms, causing their adaptation, mutation and transformation into pathogenic schtamms and oncogenes. Elimination of the latter leads to the involvement of the new (inherent) microorganisms, starting the chain reaction with unforeseen consequences.

Peptic Ulcer is one of the most relevant problems of modern gastroenterology. Supposition made by Barry Marshall on H. Pylori being the cause of peptic ulcer provoked an explosion in modern science, which later was referred to as the greatest discovery. And the majority of the specialists evaluate that as the breakthrough in the field of gastroenterology.

Statistics show that 90% of the population of the Earth is affected by H. Pylori. The scientific data on H. Pylori showed that extermination of the microbes in ulcer patients leads not only to long remission but also to a final cessation of disease relapse.

However, we can not deny the fact that the development of a disease is possible only under the influence of a complex of adversities, which reset the ratio of “protection” and “aggression» factors to the latter and may take place under the decrease of defensive forces of the organism. The first includes pepsin, pepsinogenes, katepsin D and E, as well as excess of HC1 in the stomach, when the acid neutralization in the stomach has been disturbed. Microcirculation disturbance and hypoxia contribute to a damage area formation.

Adequate blood supply is necessary for healing of any injury or damage, the disturbance of which causes hypoxia and encumbers reparation. If this takes place under local mutated response reaction of the immunology protection cells with the following disturbance of trophic microcirculation, than the scar quality is very low. Correspondingly, the quality of healing determines not only the completeness of regeneration but future prognosis as well. The quality of ulcer healing depends not only on the condition of newly formatted mucous membrane, but, what is important, on the condition of the underneath tissues, as the mucous membrane trophics depends on them, and insufficiency creates conditions for recurrence.

With reference to the pathogenesis of ulcer formation it is essential to mention that the initial superficial epithelium damages cause immune reaction, initiated by different antigens, both food and bacterial. In response epithelium starts generating anti-inflammatory cytokines, which, in their turn, attract macrophages. Microbe settlement at the place of damage takes place which leads to infiltration of “incoming” monocytes/ macrophages. The latter activate (hyperactivate) in their own turn under the influence of antigens and/or toxins with the following release of reaction capable oxygen radicals that contribute to the focal damage enlargement. The most unfavorable situation is being created at the place of the damage, when microbes, toxins and activated macrophages appear at the same place. The vicious circle is closed. In this situation even unpathogenous (but foreign) microbes entering with food will irritate monocytes/ macrophages of the aggressive factors provoking “synthesis”.

Influence on one of the chains of this process will contribute to the disruption of the pathological process and scarring of the ulcer damage. Nowadays the world medicine activity is aimed at elimination of microorganisms, including H. Pylori, applying and changing the antibiotics combination from time to time. The same effect (though to a lesser degree) would be possible to achieve by eliminating the toxin from the same circle. Our point of view is that it is far more rational to modulate (to decrease hyperactivity of the monocytes / macrophages with the following reduction of the anti-inflammatory cytokines in the place of the damage, and simultaneous growth of neutrofilic granulocytes system, locating near the place of the damage). This mechanism could be adequate to antibiotics therapy.

The quality of ulcer healing has great significance from the point of view of recurrence. And this statement deserves attention, because recurrence normally starts at the place of the scar. Restitution, i.e. complete recovery of the damaged tissue structure, is the most optimum result of any regeneration. Unlike erosion, under ulcer conditions the underneath tissues are destructed together with the mucous membrane. Type and completeness of regeneration depends on the degree of these damages. Under the inadequate ulcer healing (substitution) the necrosis area is substituted by connective tissue with disturbed microcirculation, low oxygenation at increased vessel penetration that may be one of the most important reasons of ulcer recurrence.

Quality of mucous defect healing is dependent on absence of aggressive factors and granulation tissue evolution dynamics. Maturity of the latter is possible only at its protection against the former. The newly formed epithelium can provide the most complete protection. At the same time mature granulation tissue is necessary for its migration and differentiation. Optimum regeneration results can de achieved only at synchronization of all the above processes. At acute ulcer healing the renewal of connective tissue takes place with recovery of under mucous basis and inherent mucous membrane. The ulcer may take long time to heal because of desynchronisation of those processes. Prolonged damage of granulation tissue leads to its maturity slowdown and connective tissue scar formation. Migration and proliferation of fibroblasts, actively synchronizing 1st type collagen that leads to low healing quality, takes place along ulcer periphery, all that creating conditions for the subsequent recurrence.

The leading role in extra cellular matrix recovery after the damages belongs to regeneration factors, as e.g. transforming growth factor. It stimulates protein synthesis, simultaneously inhibiting proteases synthesis through their inhibitors’ synthesis stimulation. Monocyte/ macrophages and thrombocyte (represent the sources of transforming growth factor) hyper activation, that contribute to the factor’s production, which is the reason of excessive scarring.

According to the above-mentioned, modulation of immune system cells, which are responsible for ulcer disease support, as well as ulcer defect regeneration and epithelisation, could be more perspective ulcer disease treatment than elimination of H. Pylori.

TAMERIT represents such a remedy that reversibly inhibits macrophage hyperactivity in the place of damage through the biosynthetical processes digression with the subsequent anti inflammatory cytokines decrease, oxygen consumption decrease with the subsequent generation decrease of reaction capable radicals with simultaneous microbicidic system increase and transforming growth factor synthesis with polymorphic-nucleous leukocytes.

Experimental data and numerous results of clinical experiments taking place for 6 years, proved the method efficiency. It is necessary to point out that in a majority of cases along with the short periods of ulcer healing the epithelisation of the place of damage takes place that is very important for the prognosis of the disease. It is very seldom that recurrence can take place at the same place. In certain cases if scarring takes place, the scar is small, delicate and elastic.

**PHARMACOLOGICAL EFFECTS OF TAMERIT**
   **Most of the pharmacological effects of Tamerit are due to its ability to regulate the function of monocytes/macrophages**

►  Immunomodulatory effects depend on immune reactivity of the body. In case of immune insufficiency, the preparation reactivates cytokine-producing and tumoricidal function of macrophages. Simultaneously, it restores normal function of T-cells and strengthens phagocytosis and microbicidal system of neutrophilic granulocytes.

►  Anti-inflammatory effects. In case of inflammatory diseases, Tamerit suppresses reversibly for 10-12 h hyperactivity of macrophages, reduces synthesis of pro-inflammatory   cytokines (TNF, IL-1, IL-6), nitrocompounds and reaction-capable radicals, activates production of anti-inflammatory cytokines (IL-10).

►  Anti-oxidant activities. Tamerit decreases the uptake of oxygen by activated macrophages and generation of oxygen radicals.

►  Antitumor activities.   Tamerit stimulates tumoricidal function of macrophages, cytotoxic T cells, NK cells and activates production of cytokines taking part in antitumor response (IL-1, TNF, IFN-γ).

►  Stimulation of tissue regeneration. The preparation stimulates growth of granulation and epithelization of ulcerous defects of skin and mucosa.

**INDICATIONS FOR USE OF TAMERIT**

Acute and chronic viral and bacterial inflammatory diseases of gastrointestinal tract (including viral hepatitis), urogenital and breathing systems (including tuberculosis)

Septic diseases, exogenous and endogenous intoxication

Diseases accompanied with immune insufficiency

Prevention of toxic effects of chemo- and radial therapy in patients with cancer

•                     Prevention of cancer metastases

•                     Prevention and treatment of infectious complication of surgical operations

•                     Ulcers of gastrointestinal tract and skin