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THE EFFECT OF BIOSTIMULANTS FROM THE SPLEEN ON THE ACTIVITY OF T-LYMPHOCYTES AND NATURAL KILLERS

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Summary

The work is devoted to the study of the effect of a biostimulator and its compounds with zinc on spontaneous, PHA-induced blast transformation of T-lymphocytes in patients with chronic viral hepatitis B, as well as on the cytotoxic activity of natural killers in colon cancer, and healthy donors in the in vitro system. The results showed that the biostimulator has a moderate regulatory effect on spontaneous blast transformation of lymphocytes in the in vitro system. The costimulating effect of the biostimulator of the PHA-induced blast transformation of T-lymphocytes was revealed. A significant increase in the membranotoxic function of natural killer cells in relation to K-562 target cells was found under the influence of a biostimulator after preliminary incubation of the drug with effector cells. The biological efficiency of splenic peptides increased after the addition of zinc ions to them.

Key words: *biostimulator, immunomodulator, antibodies, T-lymphocyte, metallopeptide, immunodeficiency, in vitro.*

Introduction. Thymus extracts, including thymosin, are used in the treatment of cancer, autoimmune diseases, many chronic infectious processes and others [1-6].

The most important mechanism of action of thymic peptides is the enhancement of the functional activity of T-lymphocytes, however, the multi-stage process of developing an immune response involves the activation of not only cellular, but also humoral factors of immunity, contributing to the increased production of specific antibodies, cytokines, inflammatory factors and others [6]. Natural immunity is largely determined by killer cells (KC), which play a decisive protective role in the early stages of viral aggression [7].

Among the known thymus peptides, a drug obtained from the spleen is of particular interest. To increase the immunobiological activity of peptides, we attempted to combine them with metal ions, as is the case in thymulin, which circulates in the bloodstream as a nanopeptide combined with zinc [6-8].

The aim of the work is to study the effect of splenic peptides on the functional activity of human T-lymphocytes and natural killer cells in the in vitro system.

Material and methodology. Determination of the effect of drugs on the proliferative response of T-lymphocytes.

The peripheral blood mononuclears of 32 patients with chronic viral hepatitis B aged 20-49 years served as the material for the study of lymphocyte blastogenesis.

Phytohemagglutinin (PHA) (Sigma) and concanavalin A (Con A) (Pharmacia) in suboptimal concentrations (10 mcg/ml) were used as an activator of the lymphocyte blast transformation reaction (RBTL).

Modified and unmodified peptides were added to the lymphocytes (1 million/ml) in the experimental samples at a final concentration of 0.01 mcg/ml. The tablet was

incubated at 37 ° C - 1 hour, after which the corresponding mitogen was introduced into the wells. Only mitogen was added to the control samples of lymphocytes. Mitogen was not used in the study of spontaneous blast transformation of lymphocytes. After 48 hours, 3H-thymidine was added to the samples at a concentration of 1 MCC/ml. The reaction results were taken into account 72 hours after the start of cultivation.

To quantify the effect of protein peptide compounds on the proliferative response of T-lymphocytes, the exposure index (EI) was used, which was calculated by the formula:

EI = (Pe – Ic)/Ic • 100%, where : Pe is the number of pulses per minute (imp/min) in the experiment;

Ic is the number of imp/min in the control.

Determination of the effect of drugs on the cytotoxic activity of natural killers.

The material for the study of cytotoxic activity was peripheral blood mononuclears of 30 people, including 14 healthy donors and 16 patients with colon cancer II- IV art.

The concentration of lymphocytes isolated from peripheral blood was adjusted to 1 million/ml. The membranotoxic activity of natural killer cells was determined by the ability of effector cells to damage target cells labeled with 3H-uridine 9 [8].

Transferable cells of the human myeloid leukemia line K-562 (5 million/ml) labeled with 3H-uridine: I MCC/ml were used as targets and incubated at 37 °C in a water bath for 1.5 hours. After that, the cells were washed three times with a large volume of medium 199 with 20% fetal calf serum. To prevent effector cells from reutilizing DNA hydrolysis products that included a radioactive label, target cells were incubated for 2 hours in a nutrient medium (composition: RPMI-1640 with 10% embryonic calf serum, 2 mM L-glutamine and 40 mcg/ml gentamicin) at 37 ° C and washed again once in a large volume of medium 199 with 20% fetal calf serum.

EC activity was expressed by the cytotoxicity index (CI), which was determined by the formula:

CI = (1 - the average value in the experiment (imp/min) / the average value in the control (imp/min) * 100%

Statistical processing of the results was carried out using standard methods of variational statistics with the calculation of the arithmetic mean (M), its error (m) and the Student criterion (t).

Results. It was found that the average value of spontaneous RBTL in hepatitis patients in control group 1 (without incubation with mitogen and biostimulator) was 280 ±14 imp/min with a range of individual fluctuations from 153 to 404 imp/min. In the presence of immunomodulin (control 2), the indicators of spontaneous blastogenesis significantly increased on average in the group to 351 ±26 imp/min with a range of individual fluctuations from 207 to 673 imp/min. The index of the drug's effect on spontaneous RBTL was +25.3% ($P<0.05$). The introduction of a zinc-modified peptide into the culture increased the rates of spontaneous transformation of T-lymphocytes to an average of 415±36 imp/min. With a range of individual fluctuations from 248 to 650 pulses/min. The index of the drug's effect on spontaneous RBTL was +48% ($P<0.05$ with control 2 and $P<0.001$ with control 1).

We also studied the functional activity of T-lymphocytes by their ability to enter the mitotic cycle under the influence of PHA. It was found that under the influence of lectin, the blast transformation of lymphocytes in general on control group 1 is (51.4±3.3) x 103 imp/min with individual fluctuations in indicators from 41 to 74 thousand imp/min.

In control group 2, the average value of this indicator did not significantly differ from control 1 and amounted to 57.0±2.4 thousand imp/min with individual values from 42 to 77 thousand imp/min. The biostimulator impact index averaged +11% per group.

In the main group, the average value of this indicator significantly differed from control 1 and control 2 and amounted to 65.0±2.4 thousand imp/min with individual values from 48 to 86 thousand imp/min. The average exposure index of the modified immunomodulin per group was +27% (P<0.05 with control 2 and P<0.001 with control 1).

Consequently, the modified biostimulator had a significant stimulating effect on the PHA-induced blast transformation of T-lymphocytes. depending on the initial sensitivity to PHA: in individuals with initially reduced indicators, a stimulating effect was revealed, and in individuals with an initially normal response to PHA, no effects were noted. Thus, the costimulating effect of the modified biostimulator on the PHA-induced proliferative response of T-lymphocytes was noted.

In an experiment with pretreatment of only mononuclears with the drug, similar results were obtained. Thus, incubation of effector cells with immunomodulin (without target cells) showed a significant stimulation of the membranotoxic activity of natural killers in all studied groups. In this group of experiments, 2 controls were used: preliminary parallel incubation of effector cells only in a nutrient medium (control 1) and with a peptide (control 2). Metallopeptide was evaluated in the experimental group. Thus, in healthy donors, the EC cytotoxicity index was 51.2±1.9%; incubation with a modified peptide increases these values to 65.7 ± 1.6%; preincubation with immunomodulin activates them to 58.3± 1.7%. The difference in the values of the experiment with the controls was significant (P<0.05 with control 2 and P<0.001 with control 1).

In an experiment conducted on lymphocytes of patients with colon cancer, a deep decrease in the membranotoxic activity of EC was found, which was 23.8± 1.5% (control 1). When cultured with immunomodulin, these values are 29.3± 1.5% (control 2). Preliminary incubation of effector cells with metallopeptide increases the activity of EC to 34.3± 1.3% (P<0.05 with control 2 and P<0.001 with control 1).

Discussion. The principal feature of the action of fetal peptides of the spleen is the dependence of the severity and direction of their effects on the initial state of the regulated cells, which contributes to the normalization of the processes out of balance [9, 10, 11, 12]. In particular, as our study showed, immunomodulin does not have mitogenic activity, but shows it only with simultaneous stimulation of PHA or endogenous mitogens (with spontaneous blast transformation). This indicates in favor of the costimulating effect of immunomodulin and zinc-containing metallopeptide on the lymphoblastic response. The costimulating effect of thymic peptides on blastogenesis is obviously realized through a specific receptor found on lymphocytes. Apparently, simultaneous activation of receptors for PHA and thymus peptide is an enhanced stimulus for the process of lymphocyte proliferation.

It must be recognized that when studying the biological feasibility of a particular peptide regulator, problems arose due to the pleiotropy of its action, i.e. the ability to interact with structurally different receptors localized on many cell populations and cause a whole range of diverse biological effects. It is believed that this depends not so much on its primary structure as on the stereochemical structure and the volumetric pattern of the distribution of electric charges, which allows the peptide to react with receptors of various configurations [2,13, 14].

The results of our research on the activity of natural killers showed that in the case of preliminary joint incubation of the biostimulator by effector cells, the membrane-toxic function of the EC was enhanced. We have previously shown that interferogenesis in lymphocyte culture is significantly enhanced under the influence

of immunomodulin [II]. It is possible that the effect of immunomodulin and metallopeptide on EC may also be due to the activation of T-lymphocytes through specific receptors, which, in turn, leads to an increase in the production of IFN-γ, IL-2, IL-12, IL-15, etc. cytokines with simultaneous appearance of receptors for them on other subpopulations of lymphocytes [6, 15, 16, 17]. The consequence of these influences was an increase in the membranotoxic activity of natural killer cells, since IL-2 directly, without interaction with the CD25 receptor, triggers natural killers for a cytolytic reaction [12,17]. Despite the fact that ECS do not express CD-25-γ-chains of the high affinity receptor for IL-2, cells respond to it by proliferation and increased cytolytic activity [16,17].

Earlier in the experiment, we showed a receptor-mediated effect of the biostimulator, an increase in the concentration of Ca2+ in thymocytes, which was accompanied by the redefinition of Ca2+ ions between the endoplasmic reticulum and mitochondria with an increase in the process of biological oxidation and an increase in the energy of cells [18].

Conclusion. Consequently, we have established that biostimulants combined with zinc have a regulating effect on the proliferative activity of T-lymphocytes through the interaction of their cellular recetors with mitogen and thymus peptides, which ultimately leads to the cascade synthesis of cytokines, which, in turn, modulates the proliferation of T cells and the cytotoxic activity of natural killers. An increase in the membranotoxic function of natural killer cells in relation to K-562 target cells was detected under the influence of a biostimulator after preliminary incubation of the drug with effector cells. The biological efficiency of splenic peptides increased after the addition of zinc ions to them.

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Көкбауыр биостимуляторларының Т-лимфоциттер мен табиғи өлтірушілердің белсенділігіне әсері

Аңдатпа

Жұмыс биостимуляторлардың және оның мырышпен қосылысының созылмалы В вирустық гепатиті бар науқастардағы Т-лимфоциттердің өздігінен, ФГА-индукцияланған Бласт трансформациясына, сондай-ақ тоқ ішек қатерлі ісігіндегі табиғи өлтірушілердің және in vitro жүйесіндегі сау донорлардың цитотоксикалық белсенділігіне әсерін зерттеуге арналған. Нәтижелер биостимуляторлардың in vitro жүйесіндегі лимфоциттердің өздігінен жарылғыш трансформациясына қалыпты реттеуші әсер ететінін көрсетті. Т-лимфоциттердің фга-индукцияланған Бласт трансформациясы реакциясының биостимуляторының костимуляциялық әсері анықталды. Эффекторлық жасушалармен препаратты алдын ала инкубациялағаннан кейін биостимуляторлардың әсерінен К-562 нысаналы жасушаларына қатысты табиғи өлтіруші жасушалардың мембранотоксикалық функциясының сенімді күшеюі анықталды. Көкбауыр пептидтерінің биологиялық тиімділігі оларға мырыш иондары қосылғаннан кейін өсті.

Түйінді сөздер: *биостимулятор, иммуномодулятор, антиденелер, Т-лимфоцит, металлопептид, иммунитет тапшылығы, in vitro.*

Влияние биостимуляторов из селезенки на активность Т-лимфоцитов и натуральных киллеров

Работа посвящена изучению влияния биостимулятора и его соединения с цинком на спонтанную, ФГА-индуцированную бластную трансформацию Т-лимфоцитов у больных хроническим вирусным гепатитом В, а также на цитотоксическую активность натуральных киллеров у рака толстой кишки, и здоровых доноров в системе in vitro. Результаты показали, что биостимулятор оказывает умеренное регулирующее влияние на спонтанную бластную трансформацию лимфоцитов в системе in vitro. Выявлен костимулирующий эффект биостимулятора реакции ФГА-индуцированной бластной трансформации Т-лимфоцитов. Обнаружено достоверное усиление мембранотоксической функции естественных клеток-киллеров по отношению к клеткам мишеням К-562 под влиянием биостимулятора после предварительной инкубации препарата с эффекторными клетками. Биологическая эффективность селезеничных пептидов возросла после присоединения к ним ионов цинка.

Ключевые слова: *биостимулятор, иммуномодулятор, антитела, Т-лимфоцит, металлопептид, иммунодефицит, in vitro.*

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