American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 03 ISSUE 12 PAGES: 48-55
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

Journal Website: https://theusajournals.com/index.php/ajbspi

Research Article

INFLUENCE OF MODIFIED PEPTIDES FROM THE FETAL THYMUS ON THE ACTIVITY OF T-LYMPHOCYTES AND NATURAL KILLERS IN EXPERIMENTAL VIRAL HEPATITIS

Submission Date: December 15, 2023, **Accepted Date:** December 20, 2023,
Published Date: December 25, 2023
Crossref doi: https://doi.org/10.37547/ajbspi/Volume03Issue12-09

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ABSTRACT

The results showed that immunomodulin has a moderate regulatory effect on the spontaneous blast transformation of lymphocytes in the in vitro system. The costimulatory effect of immunomodulin in the reaction of PHA-induced blast transformation of T-lymphocytes was revealed. The data we obtained on the rather pronounced interferon-inducing properties of Sanogen developed by us and its combinations with well-known inductors - Cycloferon and Betaleukin, can be of practical use in the treatment of infectious pathology, especially viral hepatitis, when the combination of hepatoprotective, anti-inflammatory, antiviral, immunomodulatory and detoxification mechanisms of action, in the absence of toxicity and side effects, will ensure the development of sanogenetic processes in the patient's body.

KEYWORDS

Toxicity, side effects, treatment of cancer, cells.

INTRODUCTION

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Thymus extracts, incl. thymosin is used in the treatment of cancer, autoimmune diseases, many chronic infectious processes, etc. [1,2,10, 28, 37]. 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 includes the activation of not only cellular but also humoral immunity factors, contributing to an increase in the production of specific antibodies, cytokines, inflammatory factors, etc. [7, 16, 22]. Natural immunity is largely determined by killer cells (NK), which play a decisive protective role in the early stages of viral aggression [26]. Among the known thymic peptides, a drug obtained from fetal sheep thymus is particularly interesting. It consists of 15 peptides. Its immunocorrective effect was shown in experiments and clinical observations, in connection with which it received the name "Immunomodulin". It is mass-produced in the state of emergency "Immunomed" (Tashkent) and approved for medical use in Uzbekistan and Kazakhstan [2].

To increase the immunobiological activity of thymic 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.

The aim of the work is to study the effect of zinc-modified thymus peptides on the functional activity of T-lymphocytes and human natural killer cells in the in vitro system and to evaluate the effectiveness of interferonogenesis and antiviral action under the influence of Sanogen, Betaleukin, Cycloferon and their combinations in the experiment.

METHODS

Determination of the effect of drugs on the proliferative response of T-lymphocytes. The material for the study of lymphocyte blastogenesis was peripheral blood mononuclear cells of 32 patients with chronic viral hepatitis B aged 20-49 years.

Phytohemagglutinin (PHA) (Sigma) and concanavalin A (Con A) (Pharmacia) at suboptimal concentrations (10 µg/mL) were used as RBTL activators. Modified and unmodified peptides were added to the lymphocytes (1 million/ml) at a final concentration of 0.01 µg/ml in the test samples. The tablet was incubated at 37°C for 1 hour, after which the corresponding mitogen was added to the wells. Only mitogen was added to control lymphocyte samples. 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 µCi/ml. The results of the reaction were taken into account 72 hours after the start of cultivation.

To quantify the effect of immunomodulin on the proliferative response of T-lymphocytes, the impact index (IV) was used, which was calculated by the formula:

$$IV = (Io – Ik)/Ik \bullet 100\%,$$

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where: Io - the number of pulses per minute (imp/min) in the experiment;

Ik - the number of pulses/min in the control.

RESULTS AND DISCUSSION

It was found that the average value of spontaneous RBTL in patients with hepatitis in control group 1 (without incubation with mitogen and immunomodulin) was 280±14 imp/min with a range of individual fluctuations from 153 to 404 imp/min (Fig. 1). In the presence of immunomodulin (control 2), the indicators of spontaneous blastogenesis significantly increased on average in the group up to 351 ± 26 imp/min with a range of individual fluctuations from 207 to 673 imp/min. The index of drug effect on spontaneous RBTL was +25.3% (P<0.05). The introduction of the zinc-modified peptide into the culture increased the rates of spontaneous transformation of T-lymphocytes to an average of 415±36 pulses/min. With a range of individual fluctuations from 248 to 650 imp/min. The index of drug 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 in terms of their ability to enter the mitotic cycle under the influence of PHA. It was established that under the influence of lectin, the blast transformation of lymphocytes in general in the 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 mean value of this indicator did not differ significantly from control 1 and amounted to 57.0 ± 2.4 thousand imp/min with individual values from 42 to 77 thousand imp/min. The impact index of immunomodulin on average for the group was +11%.

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 impact index of modified immunomodulin averaged +27% per group (P<0.05 with control 2 and P<0.001 with control 1).

In the experiment with pre-treatment with the drug only mononuclear cells, similar results were obtained. Thus, the incubation of effector cells with immunomodulin (without target cells) showed a significant stimulation of the membrane toxic activity of natural killer cells 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). In the experimental group, the metallopeptide was evaluated. Thus, in healthy donors, the EC cytotoxicity index was 51.2±1.9%; incubation with the modified peptide increases these values to 65.7±1.6%; preincubation with immunomodulin activates them up to 58.3±1.7%. The difference between the experimental

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values and controls was significant (P<0.05 with control 2 and P<0.001 with control 1).

Quantitative assessment of the induction of 1PM by Sanogen, Betaleukin and Cycloferon with separate and combined administration to experimental animals.

It has been established that Betaleukin induces IFN production within 96 hours (observation period); the maximum titer was noted after 12 hours and reached 512 units. The average titer was 118±16 units. (Fig.1, 3). Sanogen also had quite pronounced interferonogenic properties - the maximum titer was 128 units. after 24 hours with an average value of 47 ± 4 units (Fig. 1, 3).

When using monopreparations, Cycloferon was the most effective - the maximum IFN was 1024 units. after 48 hours. Under his influence, the average titer during a 120-hour observation was 295 ± 35 units. (fig.3)

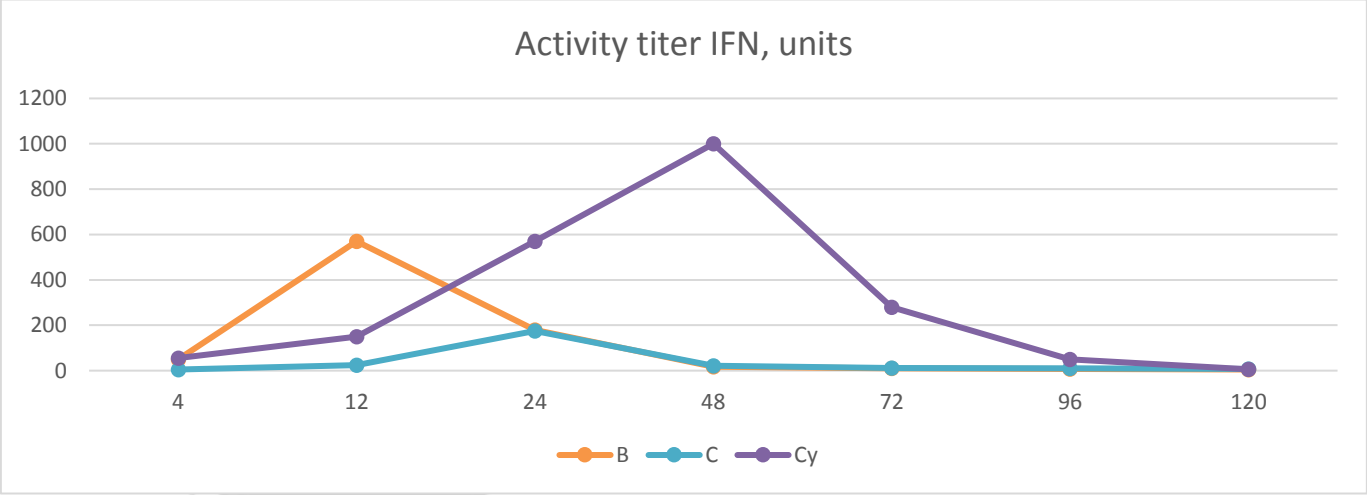

Fig.1. Dynamics of serum interferon activity in mice after a single separate intraperitoneal injection of Sanogen (C), Betaleukin (B) and Cycloferon (Cy) in effective doses: 2 μg/kg; 10 ng/kg and 4 μg/kg, respectively.

The introduction of Betaleukin with Cycloferon sharply increased the activity of serum IFN to an average of 1060 ± 80 units. The expected increase was to be 395 units. (100 units of Betaleukin + 295 units of Cycloferon), i.e. it turned out to be 2.7 times higher than the additive value. Synergy was also manifested in the accumulation of the maximum titer up to 2048 units, which is 1.3 times higher than expected (512 units + 1024 units = 1536 units). There was also a shift in the peak of IFN production for a period of 48 hours. Even 4 hours after the combined administration of inducers, the IFN activity was high and amounted to 256 units, while the serum activity remained significant and after 120 hours was 512 units. The synergistic effect for this period was 32 times higher than the additive one.

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The simultaneous administration of Sanogen and Cycloferon sharply increased the activity of serum IFN to an average of 805 ± 75 units, since the expected average increase was to be 323 units. (28 Sanogen units + 295 Cycloferon units), it turned out to be 2.5 times higher than the additive value (Fig. 3). Synergy also manifested itself in the accumulation of the maximum IFN titer up to 2048 units, which is 1.8 times higher than expected (128 units + 1024 units). The peak of IFN production was recorded for a period of 48 hours, which corresponded to the period of maximum serum activity with the introduction of Cycloferon. It should be noted that already 4 hours after the combined administration of these inducers, the IFN activity was the highest - 256 units. (Fig. 2). Despite the early and very pronounced induction, serum activity remained significant throughout the entire observation period, and even after 120 hours it was 256 units. (Fig. 2), whereas with the separate administration of Sanogen or Cycloferon, the titers were only 2 units each. and 16 units. (Fig. 1), i.e. the synergistic effect was 14 times higher than the additive one.

Therefore, with the simultaneous administration of Sanogen and Cycloferon, a pronounced synergism was found in the induction of endogenous IFN, since IFN synthesis was noted, the high activity of which is recorded in the circulating blood from 4 to 120 hours with a maximum of 2048 units 48 hours after injection, 1.8 times higher than additive action when used separately.

To quantify the activity of interferonogenesis under the influence of monodrugs and their combinations, we calculated the average values for the duration of the study for 5 days for each option (Fig. 3). It has been established that Cycloferon (295±36 units) is the most active among monopreparations. All used combinations of drugs were more powerful interferonogens compared to it. Under the influence of Sanogen with Betaleukin, the average activity of interferonogenesis was 457±45 units, Sanogen with Cycloferon - 805±56 units, and Betaleukin with Cycloferon -1060±52 units.

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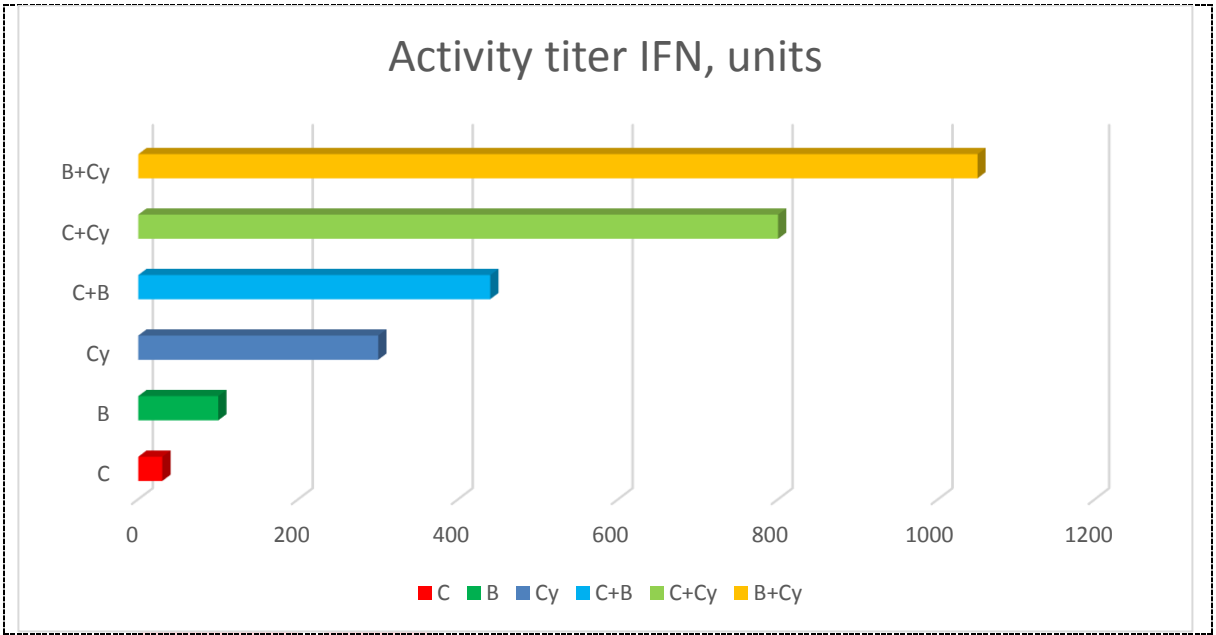

Fig. 3. Average values of serum interferon titers for 5 days after separate and combined administration of Sanogen (C), Betaleukin (B), and Cycloferon (Cy) to mice.

CONCLUSIONS

The fundamental feature of the action of fetal thymus peptides is the dependence of the severity and direction of their effects on the initial state of regulated cells, which contributes to the normalization of processes that are out of balance.

-thymic peptides combined with zinc have a regulatory effect on the proliferative activity of T-lymphocytes through the interaction of their cell receptors with mitogen and thymus peptides, which ultimately leads to a cascade synthesis of cytokines, which in turn modulates the proliferation of T-cells and cytotoxic activity of natural killers.

- pronounced interferon-inducing properties of Sanogen developed by us and its combinations with well-known inductors - Cycloferon and Betaleukin, can have practical application in the treatment of infectious pathology, especially viral hepatitis, when the combination of hepatoprotective, anti-inflammatory, antiviral, immunomodulatory and detoxification mechanisms of action

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