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Dynamics of hematopoiesis regulators content in bone marrow under the poultry embryonic tissue extract usage for the radiation-caused immune damage correction

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The aim of our study was to examine the effect of the poultry embryonic tissue extract (PETE) application at G-CSF, Flt3-ligand and IL-6 levels in the bone marrow cells supernatant of mice with radiation-caused immune disease. The PETE usage led to G-CSF levels increasing between 1st and 3rd day of observation, on the 7th day its level was at the normal rate. The comparative drug applying led to some stimulation of G-CSF levels, but compared to the PETE usage not so prolonged. During the early hours after depressive exposure under PETE treatment there was a significant IL-6 synthesis stimulation. At comparative drug applying its significant increase was registered only in the period from 16th to 24th hour. Influenced by PETE usage the Flt3-ligand level in mice with radiation-induced immune damage did not show such increase, which was observed in control animals – since the 3rd day their values were normal. At comparative drug applying the Flt3-ligand value did not reach normal rate on the 21st day of observation. Thus, due to the stimulation of the synthesis of blood formation positive regulators, which were determined in the bone marrow cells supernatant after radiation exposure, the PETE usage promoted successful and more rapid recovery of hematopoiesis, creating in turn the preconditions for effective immunogenesis.

Key words: *poultry embryonic tissue extract, radiation-caused immune damage, bone marrow, mice, G-CSF, IL-6, Flt3-ligand, ELISA.*

Динамика содержания регуляторов кроветворения в костном мозге под влиянием экстракта из эмбриональных тканей кур для коррекции радиационно-индуцированной иммунодепрессии у мышей

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Целью нашего исследования было изучение влияния применения экстракта из эмбриональных тканей птиц (ЭЭТП) на уровни Г-КСФ, Flt3-лиганда и ИЛ-6 в супернатанте клеток костного мозга мышей с радиационно-индуцированной иммунодепрессией. Применение ЭЭТП приводило к повышению уровня Г-КСФ в период с 1 по 3 сутки наблюдения, на 7 сутки его содержание определялось как нормальное. Применение препарата сравнения приводило к некоторой стимуляции уровня Г-КСФ, однако, в сравнении с эффектом от ЭЭТП, не столь продолжительному. В первые часы после иммунодепрессирующего воздействия регистрировалось значительное повышение уровня IL-6 у мышей, которым вводили ЭЭТП. В случае применения препарата сравнения значительное увеличение уровня данного цитокина было зарегистрировано только в период с 16 по 24 час. Под влиянием использования ЭЭТП уровень Flt3-лиганда у мышей с радиационно-индуцированной иммунодепрессией не обнаруживал такого увеличения, которое наблюдалось у контрольных животных – с 3 дня его значения были в норме. У группы животных, которым применяли препарат сравнения, уровень Flt3-лиганда не достиг нормы на 21 день наблюдения. Таким образом, за счет стимуляции синтеза положительных регуляторов кроветворения, которые определялись в супернатанте клеток костного мозга мышей с радиационно-ассоциированной иммунодепрессией, применение ЭЭТП способствует успешному и более быстрому восстановлению кроветворения, создавая в свою очередь предпосылки для эффективного иммуногенеза.

Ключевые слова: *экстракт из эмбриональной ткани птиц, радиационно-индуцированная иммунодепрессия, костный мозг, мыши, Г-КСФ, ИЛ-6, Flt3-лиганд, ИФА.*

Динаміка вмісту регуляторів кровотворення в кістковому мозку під впливом екстракту з ембріональних тканин птахів з метою корекції радіаційно-індукованої імунодепресії у мишей

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Метою нашого дослідження було вивчення впливу застосування екстракту з ембріональних тканин птахів (ЕЕТП) на рівні Г-КСФ, Flt3-ліганда і ІЛ-6 в супернатанті клітин кісткового мозку мишей з

радіаційно-індукованою імунодепресією. Застосування ЕЕТП призводило до підвищення рівня G-CSF в період з 1 по 3 добу спостереження, на 7 добу його вміст визначався як нормальний. Застосування препарату порівняння призводило до деякої стимуляції рівня Г-КСФ, проте, в порівнянні з ефектом від ЕЕТП, не настільки тривалого. У перші години після імунодепресуючого впливу реєструвалося значне підвищення рівня IL-6 у мишей, яким вводили ЕЕТП. У разі застосування препарату порівняння значне збільшення рівня даного цитокіну було зареєстровано тільки в період з 16 по 24 годину. Під впливом використання ЕЕТП рівень Flt3-ліганда у мишей з радіаційно-індукованою імунодепресією не зазнавав такого збільшення, яке спостерігалося у контрольних тварин – з 3 доби його значення були в нормі. У групі тварин, котрим застосовували препарат порівняння, рівень Flt3-ліганду не досяг норми на 21 добу спостереження. Таким чином, за рахунок стимуляції синтезу позитивних регуляторів кровотворення, які визначалися в супернатанті клітин кісткового мозку мишей з радіаційно-асоційованою імунодепресією, застосування ЕЕТП сприяло успішному і більш швидкому відновленню кровотворення, що створює, в свою чергу, передумови для ефективного імуногенезу.

Ключові слова: *екстракт з ембріональних тканин птахів, радіаційно-індукована імунодепресія, кістковий мозок, миші, Г-КСФ, ІЛ-6, Flt3-ліганд, ІФА.*

Introduction

The study of hemopoietic cytokines content dynamics in the bone marrow cells supernatant allows us characterizing the state of radiation-induced hematopoiesis damage at the regulatory level and to prove the potency of the poultry embryonic tissue extract (PETE) usage. Several scientists propose monitoring of the level of the hematopoietic factors: Fms-related tyrosine kinase 3 ligand (Flt3-ligand), interleukin-6 (IL-6) and granulocyte-colony stimulating factor (G-CSF) as biomarkers of a hematopoietic disorders, in particular, at radiation exposure (Mickelsen, 2012; Singh et al., 2015). The dynamic changes of these biomarkers level may serve as the predictor of duration and severity of radiation-induced disorders in the hematopoiesis and immunogenesis (Ossetrova et al., 2014). The main reason for choosing the bone marrow tissue as the medium for the biomarkers level determining is that their synthesis is predominantly tissue-specific, and consequently the maximum expression of some of them will be observed in certain "targeted" tissues (Radiation Proteomics…, 2013).

It is known, that the G-CSF stimulates proliferation and differentiation of progenitor cells to mature neutrophils, increases their survival and functional activity. These properties allow its use in overcoming of chemotherapeutic neutropenia, severe chronic neutropenia, myelodysplastic syndromes, severe aplastic anemia, and for mobilization of peripheral blood progenitor cells before transplantation (Bertho et al., 2008a). A well-known is ability of G-CSF to influence formation of reactive oxygen species via reducing the free radical production by damaged cells. Also, it is known about decreasing of the apoptosis level associated with immune depression of certain genesis under the influence of G-CSF (Bendall, Bradstock, 2014; Johns, Christopher, 2012; Zhang et al., 2013). Some scientists suggest that increased synthesis of G-CSF during treatment with certain drugs is the basis for greater animal's survival in experiments of overcoming chemotherapy and other types of hematopoiesis damage factors after-effects (Kulkarni et al., 2012).

The Flt3-ligand is an early hematopoietic cytokine, which has a paramount importance for the proliferation of myeloid, B- and T-lymphoid lineages of hematopoietic progenitor cells. In synergy with other hematopoietic factors, the Flt3-Ligand is responsible for stimulation of stem cell proliferation ($CD34^+$) and progenitor cells. In the experiment on the modeling of bone marrow aplasia in mice it has been shown that the changes of the Flt3-ligand concentration, that accompanies this pathological process, comes in the same way in tissues of organs such as thymus, spleen, bone marrow, liver, brain and blood. It has been also shown that the concentration of the Flt3 ligand is a clear reflection of the bone marrow functional state (Prat et al., 2006). The Flt3-ligand's level detection is used for biological indication of early bone marrow damage because it correlates with the severity of radiation-associated bone marrow aplasia, and variations of its concentration can be used as prognostic criteria of specified aplasia pathology (Prat et al., 2006; Radiation Proteomics…, 2013).

In our study, we have shown the greatest interest to basic fibroblast grows factor (FGFb) from all investigated cytokines produced in the bone marrow. The latter is due to FGFb presence in the studied chicken fetal tissue (Karabagli et al., 2002). A significant part of the processes, in which FGFb is involved, is unknown, probably, due to just recently expressed interest to it. However, the evidence of its role in the regulation of structuring, morphogenesis, differentiation, proliferation, cell survival and migration (Jung et al., 1994), inherently indicates the prospects of application of FGFb-containing substances for the purpose of correction of acquired immunosuppressive conditions, particularly affecting hematopoiesis and immunogenesis (Prat et al., 2005; Radiation Proteomics…, 2013).

Materials and methods

Animals

The cytokines level was determined in the supernatant of bone marrow mononuclear cells of mice in all experimental groups. The 10-weeks-old male mice were taken out from the experiment via cervical dislocation. The animals were kept in a vivarium of "Mechnikov institute of Microbiology and Immunology of NAMS of Ukraine" on a standard diet with specified conditions of animal management. Work with laboratory animals was performed according to the rules (European convention…, Strasburg, 1987).

Animals were divided into the following groups by 11 animal units in each one: I group – intact mice; II group – mice, which were subjected to external single total γ-radiation influence; III group – animals, which were intramuscularly administered the PETE in 0.1 mg/kg dose 5 times before and 1 time after irradiation. IV group – animals, which were intramuscularly administered the comparison drug ("Erbisol") in 0.1 mg/kg dose 5 times before and 1 time after irradiation.

Radiation-induced immune damage modeling

Animals were subjected to the single total gamma irradiation in a 5 Gy dose. The external single, general γ-irradiation was implemented with X-ray unit RUM-17 (USSR, Kyiv's Union Factory Production "Medaparatura") in a dose of 5 Gy within 12 minutes 30 seconds. The regime of radiation exposure was the next: one-focal distances – 40 cm, electric current intensity – 10 mA, tube voltage – 180 kV, Cu filter 0.5 + 1Al. The experimental irradiation of animals was implemented on the «Grigoriev Institute for Medical Radiology of NAMS of Ukraine" base.

The cytokines level determination by ELISA

The femoral and shin bone were subjected to the release from soft tissues. Epiphysis of received bones was separated. Diaphysis of femur and tibia bones was washed by medium DMEM, containing 15% fetal serum of cattle, 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 12 mM of L-glutamine. Mononuclear cells were isolated from bone marrow by centrifugation, washed in 5 ml culture medium for 10 min at 400 g on density gradient Lymphoprep (Sigma, r=1.077 g/cm^3). The supernatant was removed. The precipitate of each tube was resuspended in medium to a concentration 1×10^6 cells/ml. Then it was cultured for 24 hours at 37°C and 5% CO_2 in 96-well plates in an amount of 2×10^5 per well in RPMI-1640 medium supplemented with 10% fetal calf serum and 80 mg/ml of gentamicin. We investigated the spontaneous cytokines production in the supernatants, which were obtained by culturing bone marrow mononuclear cells in triplets. These cytokines were determined by ELISA on the reader «Stat-Fax 303 plus» (USA, Awareness Technology Inc.), according to the diagnostic kits instructions. In our study we have used a number of ELISA kits: «Quantikine Mouse Flt3-ligand ELISA» (USA, R&D systems) with sensitivity: 5 pg/ml and measuring range: 31.2–2000 pg/ml; «Quantikine Mouse G-CSF ELISA Kit» (USA, Invitrogen Thermo Scientific) with sensitivity: 0.5 pg/ml and measurement range: 0.5–150 pg/ml; «Quantikine Mouse G-CSF ELISA» (USA, R&D systems) with sensitivity: 5 pg/ml and measuring range: 14.1–900 pg/ml; «Mouse IL-6 Mouse ELISA» (USA, Avicera bioscience, Inc.) with sensitivity: 2 pg/ml and measurement range: 4.0–500 pg/ml.

Statistical analysis

During the statistical analysis of experimental data there was used the t-test with Bonferroni correction considering using Origin software (check for normal distribution) and Microsoft Office Excel 2003. Results are expressed as arithmetic mean (M) with a linear deviation (σ). Scale values in Fig. 1 and 2 are shown in logarithmic form.

Results and discussion

The experiment found that in healthy mice hematopoiesis was characterized by a low G-CSF and IL-6 level in bone marrow without hesitation and discontinuous variation in dynamics during 21 days after irradiation (Fig. 1). The levels of Flt3-ligand contrary were insignificant. That agrees with that normally the large number of Flt3-ligand soluble form are synthesized by endotheliocytes microvessels, stromal cells and other hematopoietic microenvironment cells, including fibroblasts (Bertho et al., 2008b; Gilliland, Griffin, 2002).

At applying of the PETE in normal non-damaged animals group (II group) transient induction of the G-CSF in bone marrow held, which is expressed in increasing its level during the period from the 12th to 24th hour observation, $p<0.05$. The peak of G-CSF content came in the 20th hour, when the level of G-CSF was greater than in intact animals (control) in 8.5 times. After usage the comparison drug there was registered a slight increase of G-CSF level on the 16th, 20th and 24th hours after the last administration, $p<0.05$. Elevated levels of IL-6 after the application of the PETE in normal control mice were recorded on the 6th to 20th hour with a peak of content on the 16th hour when excess compared to intact animals was

6.4 times. After reaching peak there was a gradual normalization of IL-6 level, and on the next time point registration (24th hour) significant increases compared to intact animals was not observed, $p>0.05$. The level of Flt3-ligand in the supernatant of bone marrow cells of control mice after the PETE application had not undergone throughout the observation period any changes. A similar situation was observed for the comparison drug. Such stimulatory effect after PETE usage, we can observe due to the ability of its components, including FGFb, to increases proliferative and differentiation activity accompanied by activation of transcription factors of some cytokines. In particular, the works of other scientists show that the induction of osteoblasts differentiation *in vitro* in culture of bone marrow stromal cells is caused by increasing Flt3-ligand production (Bertho et al., 2008b).

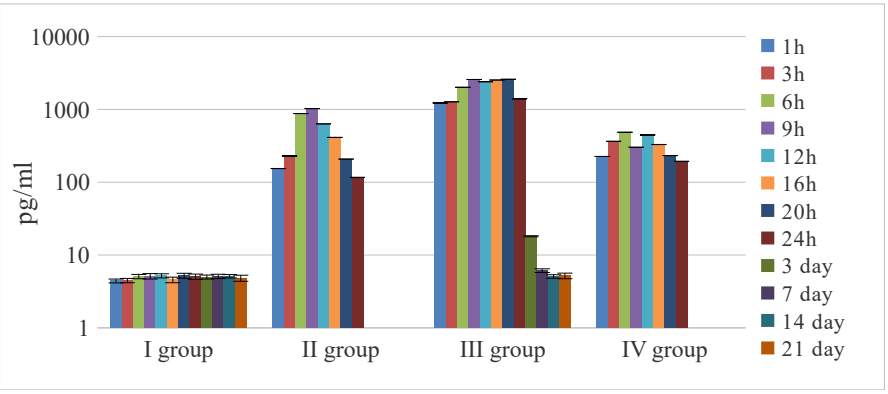

Fig. 1. The PETE usage influence on G-CSF level dynamics in bone marrow of mice with radiation-induced immunodepression (data were not detected in some groups from third until 21st day after irradiation)

In the bone marrow cells supernatant of mice with radiation-induced immune depression (IV group) there was observed an undulating elevation of G-CSF during the 1st day after exposure, with a peak that accounted for the 9th hour – 1024.18±51.09 pg/ml, $p<0.05$.

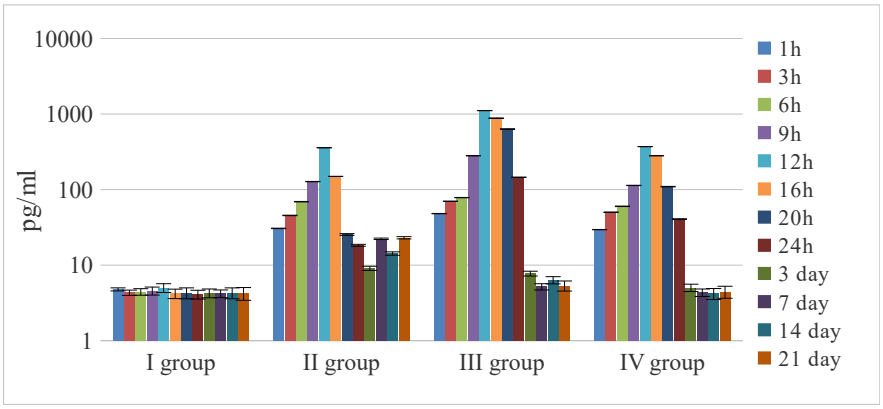

Fig. 2. The PETE usage influence on IL-6 level dynamics in bone marrow of mice with radiation-induced immunodepression

The character of the G-CSF level changing over the 1st day after the radiation exposure demonstrates the organism's reaction on violation of hematopoietic homeostasis when the induced hematopoiesis was observed, the regulation of which depends mainly on the G-CSF action and action of

the others early hematopoietic factors (Driessen et al., 2003). Starting from the 3rd day after damaging influence to the end of observation levels of the G-CSF were not recorded.

The level of IL-6 in mice with radiation-induced immune damage is characterized by a gradual increase in the period from 1st to 12th hour observation, when it reached a peak and exceeded its content in intact animals bone marrow at 87.0 times. In the period from the 3rd to the 21st day IL-6 levels remained elevated (average of 5) (Fig. 2). These data agree with the experimental data obtained in other radiation aplasia animals, which exhibit increasing levels of IL-6 in serum and in supernatants of the bone marrow cells. An increased level of IL-6 in this case is associated with increasing of IL-1 and TNF-α levels (Herodin et al., 1992).

It is known, a pleiotropic action of IL-6 includes both its pro- and anti-inflammatory properties. Thus, its increase in the early stages after the damaging effects, it is considered, targets to realization of its anti-inflammatory properties that counteract the pro-inflammatory cytokines (Chen Yong Feng et al., 2013). In addition, as is well-known, IL-6 acts as a stimulator of cell proliferation and differentiation of lympho- and miyelopoiesis lines, and as a promoter of mesenchymal precursors growth (Gilbert, Hemann, 2012). Prolonged hyperproduction of IL-6 mediates inflammation. Similarly, when hematopoiesis is damaged, IL-6 is considered as the intermediary of its reconstruction inhibition, which specific mechanism is not found yet (Fu et al., 2000).

The Flt3-ligand values in the supernatant of bone marrow cells in this group of mice was characterized by a gradual increase in the dynamics of the 1st day since the 1st hour of observation, when it was 367.15±43.53 pg/ml compared to 301.19±23.41 pg/ml in intact animals, and on the 24th hour – almost doubled and amounted to 601.23±47.84 pg/ml compared to 311.98±20.04 pg/ml, $p<0.05$ (Fig. 3). These data accord with increasing of Flt3-ligand level in plasma affected by radiation-induced immune damage *in vivo* (Kim et al., 2013). Since the 3d day we observed Flt3-ligand level decreasing compared with 24 h (1.54 times), but it still outclasses the value of intact animals by 24.2%. Since the 7th day, and throughout the period of observation Flt3-ligand level of irradiated mice was significantly lower than in intact animals with a minimum at 14th day (less than 1.53 times), $p<0.05$. As for likely explanation for changes in the level of Flt3-ligand synthesis in these conditions, in the works of some authors it has been demonstrated, that the regulation of its production is done by stimulating the synthesis of other cytokines, and not directly by ionizing radiation (Gilliland, Griffin, 2002). For example, in experiments *in vitro* TNF-α introduction to the culture of bone marrow stromal cells led to increased production of Flt3-ligand, that did not happened by the influence of independent radiation exposure.

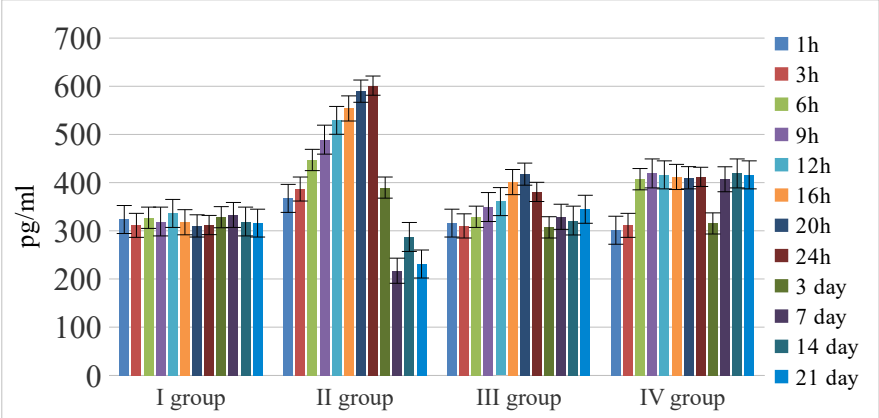

Fig. 3. The PETE usage influence on Flt3-ligand level dynamics in bone marrow of mice with radiation-induced immunodepression

The G-CSF's level in the bone marrow of mice with radiation-induced immune damage under the PETE usage multiple increased, which was recorded at the 3rd day of experiment compared with animals that were not exposed to the PETE usage. Under the influence of PETE the enhancing of this hematopoietic factor was between 16th and 24th hour surveillance, with a peak at the 20th hour (12.4 times higher than in the comparison group). This increase was sustained and not wavy. On the 3rd day

decrease was observed in intensity of the PETE stimulating effect on the G-CSF synthesis, when its level decreased compared with the 24th-hour observation in 14.5 times and amounted to 98.09±8.15 pg/ml, however, all is considerably higher than the value in the group without the PETE usage and intact animals, $p<0.05$. On the 7th day its level was at the intact animals level. So the PETE usage leads to increasing of G-CSF levels in bone marrow between 1st and the 3rd day of observation with a maximum increase to 10.6 times at 20th hour than in the animals that did not receive the PETE. Perhaps it is due to a significant increase in early-acting hematopoietic factor to the 24th hour after radiation influence, and conservation, although to a much lesser extent, of the stimulating effect until the 7th day creates a foundation for more rapid hematopoiesis recovery.

Under the reference medicinal product influence some stimulation of the G-CSF synthesis in the bone marrow of mice with radiation-induced immune damage was also incorporated. But, compared with data that were obtained under the PETE influence, G-CSF synthesis stimulation expressed only in slight increase of its value. Thus, the maximum increasing of G-CSF in mice of this group compared to animals without the PETE usage took place at the 12th hour (in 6 times). On the 16th hour observation the gradual reduction of G-CSF level was with registration minimum on 24th hour, $p<0.05$. Since 3rd day and by the end of the observation G-CSF level in mice of this group was not detected that reminded the group with radiation-induced immune depression without the use of medicinals. Therefore, when comparing the PETE influence and the reference medicinal product significant difference was found in their actions in terms of G-CSF in bone marrow supernatant.

Under the PETE application in mice from 1st h to the 3rd day the stimulation of IL-6 production in bone marrow was also observed. IL-6 level has fallen to 12th hour after damaging impact (exceeding 3.1 times) compared with the control group, $p<0.05$. In the period from 12th h to the 3rd day the gradual reduction of its level in dynamics was observed, but the significant prevalence levels were preserved compared to animals that did not receive PETE. On the 3rd day and by the end of experiment the normalization of IL-6 appeared, when in a group of mice without the PETE use the similar pattern was not occurred. Thus, already during the first hours after radiation exposure under the PETE treatment there was a significant stimulation of IL-6 synthesis, resulting in a steady increase of its levels in bone marrow.

At applying of the reference medicinal product the significant increase of IL-6 levels compared to animals without any drug administration were registered in the period from 16th to 24th hour surveillance. However, the degree of IL-6 production stimulation influenced by reference medicinal product was much smaller. It was reflected both in shorter stimulation period, and in IL-6 reliably differing level in the bone marrow from groups with PETE administration. Influenced by PETE the Flt3-ligand level in mice with radiation immune suppression in the early hours of observation was not such fast increasing, comparing with animals without the drug administration. From the 3rd day of experiment Flt3-ligand value was normal, when in the comparison group it was first raised – 307.14±30.70 pg/ml compared to 389.72±44.71 pg/ml, and on the 7th day decreased by 1.4 times compared to intact animals – 231.28±33.04 pg/ml vs 316.72±22.20 pg/ml, respectively.

In applying the reference medicinal product some stimulation of G-CSF levels also occurred, but compared to the PETE in not to the same degree and not so prolonged – on the 7th day his level was not detected. Under the influence of the reference medicinal product on the Flt3-ligand level in bone marrow of immunosuppressed mice on the 21st day of experiment its normalization was not yet reached: Flt3-ligand level was significantly higher compared with intact level, however, it was also characterized by a approximation tendency to the norm compared with the group of animals with immunodepression.

Conclusions

Thus, during the first day after radiation-induced immunosuppression modeling undulation increase was observed of G-CSF, IL-6 and Flt3-ligand in the bone marrow. A variety of cytokines production intensity may be explained by both directly radiation effect influencing on the level of their synthesis and by other cytokines, which are the regulators of hematopoietic factors synthesis, that we studied. Consequently, direct or indirect cascade-rise that took place is certainly a reflection of the response to the damaging effects. It has indicated some changes in main cellular elements functional state, which ensure a normal hematopoiesis. As it is well-known, provided the normal course of the hematopoiesis process the level of G-CSF and IL-6 in the bone marrow are just a few picograms and they have, after exposure to physiological stimulus signals, the synthesis peaks – 3–4 hours.

After applying PETE to mice with immunosuppression higher level of G-CSF was detected in the bone marrow in comparison with the control group. Normalization of its level took place on the 7th day after the immunopathological influence. When the G-CSF level in mice with immunosuppression did not

show up at all by ELISA already 3 days after exposure. This fact indicates an active hematopoietic process in hemopoietic tissue, as G-CSF in a bone marrow induces granulocytes proliferation and differentiation, also stimulates regeneration following radiation-induced myelosuppression. In addition, very important is the G-CSF ability to influence the reactive oxygen species formation level, by reducing the production of free radicals, whose role in the cells damaging is well known. Also the G-CSF ability is known to reduce the radiation-induced apoptosis, to preserve mitochondrial membrane potential and to increase the number of mitochondria, which are sensitive to G-CSF during radiation damaging influence (Zhang et al., 2013).

As we know, one of hematopoiesis depression mechanisms is part of IL-6 in the proliferation inhibition and in spreading of stromal cells, which greatly disorganize necessary for normal hematopoiesis hematopoietic microenvironment. As the main component of the hemopoietic microenvironment stromal cells surrounding the hematopoietic stem cells maintain and regulate the internal environment to stabilization, differentiation, proliferation and maturation of hematopoietic precursors by secreting a variety of cytokines. Therefore, defects in bone marrow stromal cells, probably mediated by IL-6 prolonged synthesis, which starts in this case to show anti-inflammatory properties, can lead to a deepening post-radiation crisis of the bones marrow hematopoietic function (Chatterjee et al., 2002). Thus, mice that were subjected to the damaging influence observed prolonged increase of the IL-6 level in the bone marrow. In particular, its prolonged overproduction creates a base for cytokine dysregulation during hemo- and immunogenesis damage postradiation recovery. Contrary, under the PETE usage we observed increasing IL-6 level only in the dynamics of the 1st day with normalization on the 3rd day of experiment.

Increasing of Flt3-ligand level during the 1st day after radiation exposure had changed by its declining against control in the experiment end. The PETE application prevents the fluctuations of Flt3-ligand level, when for the 1st day its level significant rising was not observed. Therefore during the recovery period there was not abnormal Flt3-ligand decreasing in the bone marrow of mice with radiation-caused damage.

The events that unfolded after the PETE application in the bone marrow, including some stimulation of the G-CSF synthesis and modulation of the IL-6 synthesis at the 1st day, and preventing the wavy changes in the intensity of the Flt3-ligand synthesis, accompany the faster normalization of kariocytes total number and, in particular, lead to faster recovery of the T- and B-lymphocytes precursors.

This effect, in our opinion, can be attributed in particular to the FGFb ability, which is a part of the PETE, to regulate the activity of bone marrow stromal cells. This assumption is based on the FGFb2 ability to play a central role in cells proliferation and differentiation regulating in bone marrow *in vivo* and *in vitro* (De Haan et al., 2003). In addition, we know that FGF2 not only directly stimulates the proliferation of hematopoietic and stromal cells in the bone marrow and indirectly modulates the various stromals cytokines production in the bone marrow (Zhao et al., 2012). FGF2 also reliably shows the ability to hematopoietic tissue protection from ionizing radiation damaging effects (Eckert, Bauer, 1998).

Thus, due to the stimulation of the positive regulators of blood formation range, which levels were measured in the supernatant of bone marrow cells, in the early hours after experimental radiation exposure, the PETE usage promotes successful and more rapid hematopoiesis recovery, creating in turn the preconditions for effective immunogenesis.

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