Condition of Immunity and activity enzymes of purine nucleotides at the rats, having exposed to the dust and radiation factor and their correction

Oralbek Ilderbayev, Raikhan Tuleutayeva, Dametken Suleymeneva, Sholpan Abralina, Assem Mahatova, Aigherim Mussina

Semey State Medical University, Str. Abay, 103, Semey, Repulic of Kazakhstan, 071400

Abstract. The results of state immunity, purine metabolism in asbestosis and combined effects of radiation. Research were carried out on white rats experimentally, by immunological and biochemical methods. Three tests series on 45 albino rats have already been carried out for the assigned task realization. In the article results in state of immunity and activity enzymes of purine nucleotides of rats in dust-radioactive influence and their correction. Established, that combine influence of asbest dust and radiation lead to disorder of functions immune system of cells, which accompanied with change activity enzymes of purine nucleotides metabolism. Revealed modulating property of phyto-medicine Be was a detected.

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Introduction

The structural and functional disorders of the tissue homeostasis, having accompanied by this or that pathology beginning and development, are always connected with the tissue integrity disorder, the various cell populations´ death and reproduction just in the lesion focus. In its turn, this means, that some universal characteristics of the destructively and reparative processes, having proceeded in the organism´s organs and tissues, have to be existed, besides the specific metabolic shifts, having caused by the ethiopatogenetic diseases factors. The enzyme systems of the free predecessors exchange of the nucleic acids and their derivatives are belonged to a number of such pathological processes characteristics. So, the immunodeficient states are connected with the enzymes activity disorder, having controlled the adenosine level in the lymphocytes: the adenosine deaminase (ADA) insufficiency results in the serious immunodeficiency, at which the number is being lowered and the T – and B – lymphocytes function is being disturbed; the 5 – nucleotidase insufficiency results in the easier immunodeficiency form, at which the B – lymphocytes functions are being left normal, but the T – lymphocytes functions are being violated [1 and 2]. Expression of the T-helper 1 (Th1) – and helper 2 (Th2)-type cytokines decreased after low doses and increased after high doses. Interleukin 6 (IL-6) reacted at early times and IL-10 at later times. IL-5 levels were consistently elevated. These data highlight the differences in the responses of different splenocyte subpopulations to low- and high-dose radiation [3 and 4].

The decrease of natural killer cell activity was intensified in the irradiated mice, showing lower than values to those of old mice. Interestingly, in irradiated mice, the absolute numbers and the percentages of natural killer (NK) cells was extremely decreased. But the absolute numbers of Th cells and cytotoxic T (Tc) cells in old mice were significantly decreased. An immunological imbalance by the whole-body irradiation of 5Gy induces to persist in the long term, resulting in the similar results with aging [5 and 6].

Significant immunological alterations noted include: (i) attrition of T-cell functions, as reductions in mitogen-dependent proliferation and interleukin-2 (IL-2) production; (ii) decrease in helper T-cell populations; and (iii) increase in blood inflammatory cytokine levels. These findings suggest that A-bomb radiation exposure perturbed one or more of the primary processes responsible for T-cell homeostasis and the balance between cell renewal and survival and cell death among naïve and memory T cells. Such perturbed T-cell homeostasis may result in acceleration of immunological aging. Persistent inflammation, linked in some way to the perturbation of T-cell homeostasis, is key in addressing whether such noted immunological changes observed in A-bomb survivors are in fact associated with disease development [7 and 8]. Purine nucleoside phosphorylase (PNP) plays a leading role in the cell metabolism of nucleosides and nucleotides, as well as in maintaining the immune status of an organism [9]. Adenosine deaminase (ADA) is involved in purine metabolism and plays a significant role in the immune system. It is important to point out that the intense presence of ADA in Leukocyte suspension (LS) highlights the relevant effects in the immune system and

in vitro cytotoxicity of MeHg exposure [10]. The catabolitic processes of the purine nucleotides exchange in the organism have the large significance for the immunological organism reactivity regulation.

And the adenylate cyclase system is also involved in the adenosine immunosuppressive system and cytotoxic action. But the cyclical adenosine monophosphate (cAMPH) can be served not only the negative, but and the positive lymphocytes function´s regulator. The cAMPH effects different orientation can be conditioned by the its content change in the various immune system cells compartments, by these compartments functions, having provided the lymphocytes biological qualities or the other cells [11].

The aim in the experiment on the animals at the combined influence of the asbestos dust and the ionizing radiation has been defined by us, to study the immune status change, and the enzymes activity of the purine nucleotides metabolism: the adenosine deaminase (ADA), the adenosine monophosphate deaminase (AMPH-deaminase) and 5´ – nucleotidase (5´ – NT) in the more sensitive tissues of the liver, the thymus gland organs, and in the blood lymphocytes. Our aim is, moreover, to find it out the immunity changes interconnection presence with these enzymes activity, and also to study the Be phytopreparation action (e.g. triterpenoid from the Betula Pendula Roth).

Materials and methods

Three tests series on 45 albino rats have already been carried out for the assigned task realization. The I – st group – the intact ones (n=15), the II – nd group – the irradiated ones in 6 Gy (Grey) dose, having primed by the asbestos dust, at the same time (n=15), and the III – rd group – the irradiated and primed ones by the dust and having received the Be phytopreparation (n=15). The II – nd and the III – rd groups´ animals have been one time irradiated during 30 days and nights before the examination on the "Teragam ^{60}Co" radio therapeutic installation (Czech Republic) in the 6 Gy dose. We have carried out the animals´ topometric and dosimetric preparation on the "Terasix" X – ray photography simulator, which is provided the correct bringing to the planned dose, before to conduct the irradiations. The asbestos dust was brought into the rats´ lungs (e.g. intratracheally) for the pneumoconiosis reproduction by the method, which had been developed by us [12]. The animals have been killed by means of the incomplete decapitation, having, preliminarily, put to sleep by the chloroform. This kind of work with the experimental animals has been conducted, in accordance with the principles of the Helsinki Declaration of the World Medical Association on the humane treatment with the animals. The study protocol was approved by the Local Ethics Committee of the Semey State Medical University, Semey, Kazakhstan with the number of No4 dated January, 2014. The immune status has been estimated on the basis of the content study in the lymphocytes´ peripheral blood and their subpopulation with the phenotypes: $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ by the method of the immunofluorescent staining of the cells with the antibodies use, having conjugated with the FITC (Fluorescein Isothyocyanate) (the "CALTAG Laboratories" Company, the USA), having adapted, exactly, for the rats´ analyses. The neutrophils´ phagocytal activity (PHA) definition, the serum immunoglobulins level of the A, M, G classes, the CIC (the circulating immune complexes), ITML (the inhibition test of the migration of leukocytes) number have been conducted.

The 5´-NT activity in the lymphocytes has been defined by the adenosine monophosphate (AMPH) hydrolysis speed up to the adenosine and the phosphoric acid, and they have been expressed in the H_3PO_4 mcmole number per 1 mg of the protein. The AMPH-deaminase and ADA activity have been defined by the methods, which have been developed by Mr. Tapbergenov C.O., by the deamination rate, and they are expressed in the ammonia mcmole per the protein mg [13]. Especially, for this investigation, the lymphocytes have been isolated from the peripheral blood, and the homogenates have been prepared from the liver and the thymus gland. The triterpenoid from the (Be) Betula Pendula Roth has been used, as the phytopreparation, and it has been prescribed by the 2,5 mg./100 gr. of the mass per os during 14 days. The immunological and biochemical indicators have been defined in the all above – mentioned groups with the statistical processing and with the Student´s criteria calculation carrying out [14].

The Results and the Discussion:

The investigation results have shown that the leucopenia is registered at the combined gamma – irradiation and the asbestos dust exposure. The lymphocytes quantity has been increased, for certain, in the experimental groups: in the II – nd group for 41,44%, in the III – rd group – 28,63%. The $CD3^+$ lymphocytes absolute and the relative quantity lowering has been registered, reliably, in the II – nd group: in 2,33 times (e.g. $p<0,001$) and 1,34 time (e.g. $p<0,05$), correspondingly. After the treatment, the $CD3^+$ lymphocytes absolute quantity rise takes its place for 65,82 % and the relative amount for 27,45% (e.g. $p<0,05$), in comparison with the group, which has been exposed to the dust and the radiation factor.

The $CD4^+$ lymphocytes absolute and the percentage quantity at the animals, which have been exposed to the dust and the radiation factor, statistically,

are being lowered, for certain: the absolute quantity – in 1,78 time (e.g. p<0,001), the relative quantity – in 1,43 time (e.g. p<0,05), but at the animals, having received the phytopreparation, the $CD4^+$ lymphocytes absolute quantity is being increased in 1,88 time (e.g. p<0,01), the relative quantity – in 1,55 time (e.g. p<0,01). It has been determined, that the dust and the radiation factor is resulted in the main indices lowering of the cellular immunity system. All these given changes have resulted in the ($CD4^+/CD8^+$) immunoregulatory index increasing, that is made up, in average, 1,83±0,07 (e.g. p<0,01), in comparison with the II – nd group 1,31±0,08, that is for 21,83%. The ITML (the inhibition test of the migration of leukocytes) results have shown, that it has been mentioned the migration index lowering down to 0,60±0,04 (e.g. p<0,05) in the III – rd group, in comparison with the II – nd group. It is connected, possibly, with the Be preparation influence – the $CD3^+$ cells lymphokine producing capacity has been increased. The B – lymphocytes absolute and relative quantity were rushing to the normalization under the influence of the phytopreparation, in comparison with the II – nd group. At the same time, the statistically significant immune complexes are increased up to (1,56±0,10 standard units (e.g. p<0,01)) in the group, having received the phytopreparation, has been registered. The phagocytal activity (PHA) of the blood cells at the animals of the II – nd group has been made up 31,24±2,76%, the increase, for certain, up to 39,61±2,55% (e.g. p<0,05) has been registered in the III – rd group. The IgA common level in the blood serum, at the animals of the II – nd group has been reliably lowered (2,01±0,17 gr./l. (e.g. p<0,05)). It has, moreover, been revealed the IgA level increase in the III – rd group. It has been registered the reliable increase in the II – nd group (6,49±0,57 gr./l. (e.g. p<0,05)) at the IgM common level research. The statistically IgM level lowering, for certain, takes its place in the III – rd group (e.g. p<0,05) just after the herbal therapy. The IgG level has been increased in the II – nd and the III – rd groups, in comparison with the intact group.

Having generalized the received results, it is quite possible to state, that have been revealed the significant changes, which have been characterized by the leucopenia, the $CD3^+$, $CD4^+$, $CD8^+$ quantity lowering, the CIC (the circulating immune complex), PHA (the phagocytal activity), and IgA levels, and also by the $CD19^+$ quantity, and the IgM and IgG levels increase at the experimental animals, having exposed to the dust and the radiation factor. The almost all indications normalization takes its place, having increased the organism´s protective reaction just in the adaptation mechanisms, having possessed the immunomodulating property at the Be phytopreparation introduction.

The enzymes activity state of the purine nucleotides cycle, as at the dust and radiation exposure, well as at the phytopreparation introduction has also been studied. In the first place, the ADA activity is being defined by the T – cells content, whereas the 5´ – NT activity is being depended from the B – lymphocytes. The ADA activity sharp lowering, the purine nucleoside phosphorylase (PNPH), and the 5´ – NT in the lymphocytes of the chronic leucoses patients, is testified to on the processes slowing down of the purine nucleotides decomposition, and it also promoted to the toxic for the lymphoid cells of the metabolites accumulation – e.g. the adenosine and the deoxyadenosine. Their concentration increase in the lymphocytes short – term culture of the chronic leucoses patients under the ADA inhibition conditions is caused these cells lysis. As the research results have shown, it has been observed the ADA enzyme activity increase at the animals in 1,79 time (e.g. p<0,001), and the $5'^-$NT and AMPH-deaminase enzymes activity, for certain, has not been distinguished just from the benchmark, from the side of the purine exchange in the thymus gland at the dust and radiation exposure. It has been registered the reliable all investigated enzymes activity increase in the thymus gland cells just after the medical treatment. Thus, the enzymes stimulation and activation of the purine exchange take its place against the background of the medical treatment for the balanced action of the immune system components.

As the researches have shown, the AMPH-deaminase activity factor is not being undergone the special changes in the blood lymphocytes at the dust and radiation exposure. The 5´-NT activity is being increased in 6,33 times, and the ADA is, on the contrary, being lowered almost in 1,7 time. These changes are being promoted to the adenosine and deoxyadenosine quantity accumulation, which in the increased concentrations are able to exert the cytostatic and cytological effect upon the lymphoid cells [2]. It has been registered the positive changes of the enzymes activity against the background of the medical treatment, that is in the increase direction: the ADA activity has been increased for 59,09% and AMPH-deaminase – for 56,25%. The research results have shown, that the ADA, $5'^-$ NT, and AMPH-deaminase enzymes activity in the liver at the animals just after the dust and radiation exposure has been increased up to 0,452±0,035 (e.g. p<0,001), up to 0,044±0,004 (e.g. p<0,05), and up to 0,229±0,022 (e.g. p<0,001), correspondingly. It has been registered the subsequent enzymes activity increase, which is being resulted in the metabolism activation of the purine nucleotides at the medical treatment.

It is quite known, that the cytoplasmic enzymes of the adenosine deaminase ADA and PNPH are being

catalized the consequent stages of the adenosine and deoxyadenosine degrative transformations, which are the intermediate metabolic exchange products of the purine nucleotides. The superficially – membrane 5´ – nucleotidase is dephosphorylized the extractocellular AMPH with the adenosine formation, which is easily able to be transported inside the cell.

Thus, all these enzymes are being taken their part in the maintenance of the metabolites intracellular balance of the purine exchange – that is, the adenosine and deoxyadenosine, which in the increased concentrations are able to exert the cytostatic and cytological effect upon the lymphoid cells [2]. The pathogenetic mechanism has been defined at the molecular level with the indication of the specific deficient enzyme at the IDS (immunodeficiency states) series: the ADA one at one from the IDS and PNPH or the transcobalamin II forms at the IDS one, having accompanied by the megaloblastic or hypoplastic anaemia. The defects at the ADA and PNPH enzymes level are being broken the adenosine metabolism. These enzymes defects are being blocked the hypoxanthine production, so the adenosine, ADP, and ATPH excessive accumulation is being taken its place just in the tissues, that is being blocked the T – cells maturation, by the unknown reasons. It is quite possible to use the metabolism enzymes of the purine nucleotides in the functional condition analysis of the immune status and also in the immune reaction adequacy [15]. Thus, it has been registered in our experiment that the dust and radiation factor is being brought to the functions disorder of the immune system cells, having changed their activity that is being accompanied by the activity lowering of the exchange enzymes of the purine nucleotides. The dysfunction state of the immune protection system is being appeared, that it is quite able to intensify the adaptative mechanisms disorders. The immunomodulatory quality of the Be phytopreparation has also been revealed.

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Corresponding Author:
Dr. Ilderbayev Oralbek
Semey State Medical University, Kazakhstan
Abay str., 103, Semey, 071400, Kazakhstan

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