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[1]N.B. Akhmatullina*, [2]Sh.A. Beisembayeva

[1]National Academy of Sciences of the Republic of Kazakhstan, Almaty, Kazakhstan
[2]S.D. Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan
*E-mail: nazken1@gmail.com

Biological dosimetry and unbiased assessment of detrimental radiation effect on humans

Abstract. Biodosimetry is a modern method of immediate and unbiased assessment of the effects of different radiation doses on humans. Unfortunately, many radioecologists ignore this major purpose, considering the information of values of physical analytical changes to be sufficient. This short excursus on the substantiation of the biodosimetry role was made in order to remind radioecologists that the issue of radiation danger must be primarily based on the assessment of its negative effect on the human genetic apparatus. The relevance of biodosimetry has increased upon identification of a new effect – radiation-induced genome instability resulting in remote consequences demonstrated as de novo through the number of subsequent generations. Any extrapolations of the findings of physical environmental measurements, or even the data obtained in different animals, are not acceptable. Each of them has its own role in the assessment of radiation effect. We think that protection of human health and life is of highest importance.

Key words: biodosimetry, modern method, radiation, human health.

Introduction

Today biodosimetry is one of the most topical problems of radiation ecology which is developed and improved on the basis of the achievements in radiation genetics and radiobiology. The main task of the radioecology can be summarized as development of methods for unbiased assessment of detrimental radiation effects on humans and relevant verification of the radiation dose assessments obtained in other ways or in deficiency of reliable individual physical dosimetry. Such assessment has long been based on physical analytical methods. Moreover, there was a tendency to substitution of biodosimetry with determination of the quantity of radionuclides in the human body or radioactivity of biomaterial by methods of gamma spectrometry and EPR analysis. This distorts the principle of biodosimetry; therefore we cannot agree with the statement of Professor I.B. Mosse that simple registration of radioactivity in the body using physical or chemical methods does not reflect the essence of the biological effects [1]. Moreover, these methods disregard the mechanisms of interaction of cells or subcellular structures (especially, genetic ones) with different doses of radioactive factors.

Main body

The main mechanism of negative radiation effect is associated with primary cell genetic apparatus damage by radiation. That is why the biodosimetry methods recommended by the WHO and IAEA are based on identification of changes of the spectrum and frequency of chromosome and chromatid damages [2, 3, 4].

These methods enable to conduct fundamental studies of the dependency of biological effects on the radiation dose. It is also connected with addressing issues relating to control of radiation effect on human health and radiation safety, in general. Such objectives are of high importance in the current context of radiation situation prevailing in different regions of the world and amid the increasing technogenesis. There are millions of people in the world who were or are exposed to radiation due to their profession or due to residing at radiation polluted territories. A considerable number of people living in Kazakhstan should be included into this group.

In the broad sense of the word, currently a multitude of methods using biological indication of radiation damage are associated with biodosimetry. These are grouped as follows:

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• cytogenetic methods which are based on determination of the frequency of changes in the structural disturbance of chromosomes in peripheral blood or bone marrow cells;

• molecular genetic methods based on identification of gene mutations (GPA-TCR, HPRT-HLA, Hb and others);

• haematological methods based on measurement of changes in the number and proportions of peripheral blood formed elements;

• biochemical methods based on assessment of changes in biochemical properties of blood and urine;

• immunobacteriological methods based on assessment of changes in immune reactivity of a radiation-exposed body and composition of microflora of the external tissues and intestine;

• biophysical methods based on registration of postradiation changes in biophysical properties of biological molecules. These methods may conditionally include the method of EPR-dosimetry of enamel of teeth removed.

• epidemiological methods based on medical statistics data on the typical structure of increased morbidity in people living in radiation polluted regions. However, in the absolute majority of cases no direct evidences of involvement of radiation into the etiology and pathogenesis of such pathologies have been found.

Biodosimetry based on cytogenetic methods has gained widespread acceptance in practice. The molecular genetic analysis has also proved its potential over this period of time. This work is devoted to discussion of information relating to these two methods the author-geneticist considers the most congenial and acceptable.

The foundation for using cytogenetic methods for biodosimetry was laid by M.A. Bender and P.C. Gooch [5]. They proceeded from the assumption that chromosome damage is the earliest reaction of cells and subject to precise quantitative registration. They showed that radiation-induced chromosomal aberration can be studied in any cells division of which can be caused in vitro by adding an augmenter. Peripheral blood lymphocytes which are quite uniformly distributed all over the body and circulate in all tissues have practically identical cell division cycle (G_0 or G_1), thus ensuring synchrony in cell cultures, proved to be the most promising cells. It was also established that there is a distinct correlation in this system between cytogenetic damages and radiation doses in vitro and in vivo in terms of types and number of inducted aberrations (6, 7). Therefore they considered it logical that the number of inducted aberrations registered in human blood lymphocytes determines the average radiation dose received. Since then «karyological test» has been recognized as the major «biological dosimeter» [8]. The major advantage of the test is the high commonality of human blood lymphocyte cultivation technique and preparation of chromosome preparations in all laboratories of the world, thus ensuring adequacy of comparison of findings. It can be easily used for building a calibration curve which is an important chain in biodosimetry.

Thus, the fundamental biodosimetry principles were developed at the early stages of biodosimetry introduction into assessment of the radiation effect on humans. That was also when many other biodosimetry process conditions were studied. It was established then that calibration curves can be built using the percentage of damaged cells, total number of chromosome aberrations, paired fragments, and the amount of dicentrics and rings in radiated cultures of peripheral blood lymphocytes [8]. Advantages of the use of dicentrics and rings were demonstrated. They are easier to identify and belong to radiation exposure markers. Furthermore, reliable data were obtained evidencing of approximate coincidence of the doses determined using biological and physical dosimetry methods. So, Zh. Lejeune et al. demonstrated [9], that the doses calculated using dicentrics + rings in the group of those accidentally exposed to γ-photons varied from 28 to 49 rad, or from 30 to 50 rad on the basis of physical dosimetry data. Many other evidences of such approximation have been obtained later [10]. A series of circumstances affecting the accuracy of the findings has been studied. The duration of cultivation of lymphocytes at the time of registration of the induced aberration frequency required special attention. It was found that gradual elimination of a part of cells with aberrations instable occurs in the process of further division [11]. Therefore UNSCEAR and WHO recommended analyzing chromosome aberrations in the first division lymphocytes or exposition whole blood to radiation at 37^0C with relatively higher power for building calibration curves and for examination of persons exposed to radiation [2, 12].

Choice of mathematic model is essential for building calibration curves. Dosimetricians primarily use four types of such models on the basis of different equations: linear – $Y= a^o + bD$, quadratic – $Y= a^o + cD^2$, linear quadratic – $Y=a^o + bD + cD^2$, and power law – $Y= a^o D^{n,}$ where Y is a value of the effect under consideration, D is the radiation dose, a^o, b, c are parameters of the models [7]. Ac-

cording to A.V. Sevankayev and A.P. Nassonov, the requirements of the specified types of cytogenetic indicators are met in case of linear quadratic model [7]. This may be due to the fact that the frequency of two-break aberrations (dicentrics and rings) increases with the dose increased approximately in quadratic dependency. These equations underwent various modifications subject to the nature of radiation, e.g. the dose, the prolongation degree, and the size of radiation area, the sampling size of the cells analysed, aberration frequency, use of additional analytical methods, etc. [13]. In principle, individual researchers must use their own calibration curves (i.e. obtained in their laboratories) for biodosimetry.

Further improvements of cytogenetic biodosimetry were driven by the scientific and technical progress in the area of molecular genetics and cytogenetics. So, development of the chromosome differential staining method (G-banding) facilitated identification of aberrations which could not be isolated by solid staining method, i.e. translocation and inversion [14-16]. Since these aberrations belong to the class of stable aberrations, the method is recognized promising for retrospective diagnostics. Another advantage of the method is the possibility of isolation of thin structural changes in each chromosome. However, differential staining of cytogenetic preparations is labour and time consuming. Another more promising approach is the use of fluorescent in situ hybridization (FISH) by selective staining of homologous chromosome pairs using molecular probes specific to certain DNA sequences. This method reduces significantly the time of the analysis and the number of metaphase analysed. The development of the market of commercial molecular probes contributes to improvement of the method by increasing the analysis procedure commonality. However, FISH-method based biodosimetry belongs to the category of expensive laboratory techniques requiring special equipment and commercial preparations, and, though being inferior to the generally accepted analysis of the yield of dicentrics, it is still the only method for retrospective evaluation of the dose ulteriorly after exposure to radiation.

Rapid development of biodosimetry problem has been observed since the early 90s of the last century in connection with the disaster at the Chernobyl Nuclear Power Plant (CNPP). That time a pressing need arose not only for unbiased assessment of consequences of the effects of radioactive emissions and fallout on humans and their living environment, but also for immediate analysis of the dose dependence of the identified effects. In order to address these objectives many international programmes were established (there were 16 such programmes from 1991 to 1994). Many institutions and healthcare facilities from both Western (England, the Netherlands, Germany, Greece, Italy, and other) and Eastern (Russia, Ukraine, Belorussia, and other) countries were involved in those programmes [17]. Initially all of them used the method of analysis of dicentrics for biodosimetry. They analysed various categories of people exposed to radiation – participant in emergency clean-up, people evacuated from Pripyat, who stayed to live in relatively clean districts. Physical dosimetry data were known in the absolute majority. Some laboratories focused on children from the radiation-exposed populations. In their work they used common calibration curve described by the equation $Y=C+\alpha D+\beta D^2$, where Y – frequency of dicentrics, C – background incidence, D – dose (g). It corresponds to the linear quadratic model. The western group of laboratories was coordinated by Dr. D. Lloyd; the eastern group was coordinated by Professor A.V. Sevankayev.

Later, on the basis of the common opinion that the half-life of dicentrics is about three years (though this is not always the case), further studies included analysis of stable aberrations able to undergo cell division and persist in the body for many years. It is assumed that stable aberrations in peripheral blood lymphocytes are products of division of stem cells in which they were initially induced.

Organization of such large-scale studies of the problem of consequences of the accident at the CNPP with engagement of a wide range of laboratories in the world became possible due to the mentioned strict commonality of cultivation of blood lymphocytes and preparation of chromosome preparations. Nevertheless, the project participants had mutual visits in order to share experience and divide functions. So, the cytogenetic preparations prepared in Obninsk were subject to the classical analysis as well as G-banding and FISH analyses in laboratories located not only in the CIS countries but also in western countries. Comparison of the findings showed no significant differences for all parameters studied. Such adequacy is an incontestable evidence of reliability of cytogenetic dosimetry. Thereafter, this fact contributed to quite successful development of international contacts connected with studying a wide range of various issues of modern radiation biology being addressed using cytogenetic methods.

Another group of biodosimetry methods connected with genuine mechanisms of action of radiation is biodosimetry on the basis of molecular

genetic methods. It is known that along with the chromosome structural mutations, gene mutations also occur in the cells of radiation-exposed organisms. Currently, five types of mutations induced in the loci of control of haemoglobin (Hb), glycophorin A (GPA), hypoxanthine guanine phosphoribosyl transferase (HPRT), and T-cell receptor (TCR) are used to measure radiation damage. They can also be classified as methods of dosimetry in the early (TCR, HPRT, Hb, HLA mutations) and remote (GPA mutation) periods after exposure to radiation (18). The mutations which have been studied relatively comprehensively are TCR and HPRT mutations [14].

Mutations at TCR-locus may be recommended for early dosimetry of radiation effects for a number of circumstances. First, they originate in mature lymphocytes and eliminate with renewal of lymphocytes. It is assumed that the half-life of TCR- mutant cells is 3 years. Second, the frequency of such mutations correlates clearly with the frequency of unstable chromosome aberrations identified within the same period. Rapidly eliminated (within several months) mutations at HPRT-locus may be included into the same category. However, their use in biodosimetry is impeded due to the labour-intensive and time-consuming identification process (1-2 weeks).

Currently, the most topical trend is development of new retrospective dosimetry methods. A number of studies offer using selectively neutral GPA-locus gene mutation for this purpose [19]. Theoretically, it could be an intravital dosimeter, because it originates in long-lived bone marrow cells, i.e. stem-type cells. However, there are some restrictions for practical use of this test. They include individual variability of frequency of mutant cells among control subjects and subjects exposed to the same dose of radiation, reduction in the method sensitivity in case of prolonged and chronic exposure to radiation (at least, in case of doses less than 1 g), possibility of analysis of only the donors with heterozygous locus, making only a half of the population, lack of specific marker of radiation effect. The matter is that the gene mutation rates can increase not only from the ionizing radiation effects, but also from other genotoxicants.

Conclusion

The modern approach to biodosimetry connected with the requirements to organization of control over the health of persons exposed to radiation must be based primarily on identification of specific radiation-genetic effects at the cellular and molecular levels. Currently, the most developed analyses are cytogenetic analysis of structural damage to chromosomes in peripheral blood lymphocytes, study of DNA-replication disorders identified by in situ hybridization (FISH analysis) and search of different types of point mutations. All these methods are based on the basic mechanisms of detrimental effect of radiation on living organisms, i.e. damage of the primary DNA structure with consequent damage of its macromolecular organization with subsequent transformation into supermolecular changes in the cell genetic apparatus. The intermediate stages of these transformations and the spectrum of radiation-induced damage of chromosomes have been comprehensively studied in accordance with the fundamental research plan of general and molecular genetics and radiobiology. These developments have not changed the essence of biodosimetry, since they were only related to modification of the analysis protocols or orientation to the frequency of change of certain types (stable or unstable) of aberrations using both classical methods and fluorescent *in situ* hybridization (FISH). The results of numerous comprehensive examinations of large contingents of people with different radiation scenarios serve as evidence of the fact that chromosome and gene mutations are the main criteria of pathogen response of the body to radiation [17, 22, 23].

For Kazakhstan, the use of biodosimetry is of special relevance due to the absence of a uniform concept for unbiased evaluation of the detrimental effect of the radioactive pollution accumulated in the environment for many years. The matter is that this problem is being addressed by specialists of different profiles, and the results are systematized on the basis of the methodology relevant to only their own specific needs. There is no actually functioning coordination of all these studies in the country. So, physicists, who consider themselves to be the major developers in the country of all issues relating to radiation effects, define biodosimetry only as determination of the number of radionuclides in the human body or radioactivity of biomaterial by γ-spectrometry and EPR-analysis. It is uncontroversial among the radiobiologists that this approach is wrong. The data obtained using these methods require verification with subsequent conduction of genuine biodosimetry, and the method, if necessary and feasible, may be used just as a part of a comprehensive programme. Therefore our purpose for preparation of this review was an attempt to draw attention to the fundamental basis of unbiased as-

sessment of the radiation effect depending on the dose or without it. Cytogenetic and molecular genetic effects are reasonably considered to be the most indicator of immediate effect of radiation on humans all over the world.

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