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Research Article

Comparison of acute and fractionated irradiation of viral cell culture

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ANNOTATION.
The aim of this study was to research the optimal modes of decontamination of cell culture media used for virus reproduction by the method of fractionated irradiation. Various cell culture media were used in this study. To simulate the artificial cell contamination we used bacteria and viruses.The results of cytological studies have shown that exposure of MDBK cell cultures in a wide dose range (from 0.5 to 10.0 Gy) and in the various forms (single and double) provided a multidirectional nature - small doses (0.5 - 1 Gy) stimulated and large doses inhibited the growth and development with increasing destruction of the irradiated cells. The most appropriate of the tested variants of radiation exposure to cell cultures appeared to be a cell fractionated irradiation at a dose of 6.0 Gy, which consisted of double exposure with 3 min interval. The cell irradiation in a small dose (0.05 Gy) at the beginning and in a high dose (5.95 Gy) at the repeated irradiation stimulated the growth, reproduction and proliferative activity of the MDBK cell line. The results of cytogenetic studies have showed that such irradiation mode had an inhibitory effect on the process of chromosome disorder; it has been marked a tendency to reduce the chromosomal aberrations in the form of chromosome bridges, fragments and breaks.

Keywords: contamination, chromosomal aberrations, radiation biotechnology, radio stimulation.

INTRODUCTION.

Taking into account the divergence of literature data on the fact that the irradiation of food products and culture media in large doses causes a change in nutrient and growth properties [1], on the one hand, and a full absence of changes in the mentioned objects after irradiation [2], on the other hand, we have carried out the following series of experiments in order to study changes in the biological properties of the irradiated culture media. At the same time we have also taken into account that the sterilizing doses of γ-rays 0.1-6.04 Gy do not alter the biological properties of vaccines, serums and various culture media, and with increasing doses

from $10x10^4$ to $50x10^4$Gy it occurs a significant destruction of the primary and secondary protein structure, radiolysis of amino acids, polysaccharides, mucopolysaccharides, lipids with an appearance of toxic (oxidized) acetaldehyde radicals, is butyric, butyric acids, hydroxyquinones, formaldehyde, malonicdialdehyde, mercaptan, carbonyl compounds and free fatty acids [3,4]. An increasing content of radiation induced toxic products of radiolysis in the irradiated culture media could have a negative impact on the physical-chemical, biochemical and growth properties of serums, reflecting both the survival of cells cultured on them and their proliferative activity [5,6].The discovery of such effects of ionizing radiation as a decrease in sensitivity to repeated exposure (adaptive response) or an increase in radio sensitivity, dictates the need for studying the mechanisms of formation of long-term effects of radiation exposure. A number of researchers [7] believe that the mechanisms of changes in cellular radio sensitivity are closely linked with the mechanisms of induction and implementation of different exposure effect - the radiation-induced genomic instability [8]. This radiobiological phenomenon is manifested in the fact that some of the cells, having survived after irradiation, can produce functionally altered progeny, in which there are chromosomal aberrations and genetic mutations with a high frequency over many generations, leading in some cases to an increased cell death [9]. The pre-exposure in low doses of plant, animal and microbial cells increases their resistance to subsequent radiation with lethal doses, providing a significant influence not only on their survival, but also on the reproductive function of cells [10].

MATERIALS AND METHODS.

In our paper we applied various culture media used for cell culture: sterile normal bovine serum (NBS), fetal bovine serum; Hanks balanced solution under the standard recipe pH 7.2-7.4; Eagle medium MEM (pH 7.5-7.6) with glutamine (NGO "Vector", Russia), synthetic medium 199 (NGO "Vector", Russia); 0.5% lactalbuminhydrolyzate solution on Hanks solution (NGO "Vector", Russia); trypsin solution, trypsin-Versene solution. The virological studies to determine the cytopathic effect (CPE) in the cell cultures were carried out using a vaccine strain TK-(VIEV)-B2 (VIEV, Moscow), infectious rhinotracheitis virus (IRT). The proliferative activity of the cell cultures was determined by microscopy and calculation of a proliferation index in accordance with the conventional method. The cell cultures were exposed to gamma-rays in various doses. Sterility of the cells, culture media, blood serums, solutions, viral materials was determined by inoculation on such media as beef-extract broth, beef-extract agar, Kitt-Tarozzi, Saburo. The irradiation of artificially and spontaneously contaminated culture media was performed on gamma plant "Issledovatel" IN-1 (Russia) with ^{60}Co radiation source at doses from $1x10^3$ to $2.5x10^4$Gy. The virus titer was calculated according to Reed and Menchu and expressed in logarithms TCD (tissue cytopathic dose)$_{50}$/cm^3. The immunological competence of viruses grown on irradiated culture media and reproduced on radio stimulated cell cultures was determined by serologic method by setting the hem agglutination inhibition test (HAIT) by the standard method.

RESULTS.

Taking into account the data available in the radiation cytology on the radiostimulative action of fractionated irradiation on the animal, plant and microbial cells, we carried out the following series of experiments to study the possibility of stimulating action of γ-rays on the MDBK cell culture by repeated irradiation at high doses of ionizing radiation. The single and contacting cells grown by standard method on MEM medium with 10% NBS supplemented with penicillin and streptomycin at 100 U/ml were subjected to doubling irradiation by the following scheme: initially at a dose of 0.05 Gy, then 3 minutes later at a dose of 5.95 Gy (total dose - 6.0 Gy). The results of experiments on the cell survival, depending on the irradiation dose and options, are presented in Table 1.

Table 1.MDBK cell survival on the 4th day after a single and double irradiation

culture irradiation option and dose	number of sells survived, %	
	monolayer	suspension
Non-irradiated (control)	98.8±0.2	97.7±0.3
single dose of 0.05 Gy	99.9±0.05	99.8±0.1
single dose of 5.95 Gy	97.1±0.07	89.1±0.3*
single dose of 6.0 Gy	96.3±0.1*	87.3±0.5*
single dose of 7.0 Gy	85.7±0.3*	79.5±0.1*
double dose of 0.05 Gy, and 3 minutes later - at a dose of 5.9 Gy	99.9±0.1	99.8±0.05
double dose of 0.05 Gy, and 3 minutes later - at a dose of 6.0 Gy	99.8±0.07	99.7±0.1
double dose of 0.05 Gy, and 3 minutes later - at a dose of 7.0 Gy	98.3±0.01	97.1±0.05

* – $P < 0.05$.

It is found that the fractionated (pre-dose of 0.05 Gy, and repeated - at a dose of 5.95 Gy) exposure has had a radio modifying effect both on single (suspension) and contacting (monolayer) cells of MDBK cell cultures - a survival of doubly irradiated cells has been higher than the control one in both culture methods. Thus, the pre-exposure of cells in low dose (0.05 Gy) has radiation-sensitizing effect, which leads to the development of cell resistance to repeated radiation at higher doses (5.95, 6.0 Gy) with an increase in their survival.Considering that an increase in the cell survival exposed to low doses of radiation could be reflected on their reproductive capacity in the population, we carried out the following series of experiments on studying the effect of double exposure on the dynamics of cell growth against the background of double exposure. The results of studies carried out are presented in Table 2.

Table 2. Dynamics of accumulation and proliferation activity of doubly (0.05 Gy, 3 min interval - 5.95 Gy) irradiated cells in the monolayer and suspension

cultivation time, h	cell concentration (CC) ($x10^6$ cells/cm^3) and proliferation index (PI)					
	control		monolayer		suspension	
	CC	PI	CC	PI	CC	PI
24	0.84±0.03	1.9	1.28±0.05*	2.1	1.23±0.07*	2.0
48	1.17±0.10	2.1	2.01±0.09*	3.2	1.99±0.13*	2.7
72	1.21±0.09	2.3	1.73±0.05*	2.7	1.57±0.15*	2.5

Cultivated concentration - $0.4x10^6$ cells/cm^3, * - $P < 0.05$.

The table data shows that the maximum accumulation occurs after 48 hours of culturing the doubly irradiated cells in a monolayer at a dose of 0.05 Gy and 5.95 Gy with the interval of 3 min between irradiations, when their concentration is $2.01\pm0.09x10^6$ cells/cm^3 with a proliferation index of 3.2. The maximum concentration of cells irradiated in suspension at the indicated doses also occurs after 48 hours of culture, constituting $1.99x10^6$ cells/cm^3 with a proliferation index of 2.7.Thus, the pre-exposure of cells in low dose (0.05 Gy) and repeated irradiation at a dose of 5.95 Gy with the interval of 3 minutes, has a stimulating effect on the growth and development of MDBK cell culture, increasing the concentration of cells in 1.77 and 1.70 times ($P < 0.05$) with a proliferation index of 3.2 and 2.7 respectively compared to 2.1 in the control.In view of the literature data that the long-term effects of cell radiation might have a multidirectional nature, we carried out the following series of experiments to study the extent of chromosome (genome) damage, induced by irradiation in adapting (small) dose of MDBK cells at different times after stimulating the cells to division. The study results of cytogenetic changes in MDBK cells irradiated in test doses by γ-rays under the long-term culture conditions are shown in Table 3.

Table 3. Cytogenetic characterization of MDBK cells irradiated at test doses (0.05 Gy, 3 min interval - 5.95 Gy) in the long-term culture conditions

passage	number of cells with chromosomal aberrations (%)					
	control			irradiation at a dose of 0.05 Gy, 3 min interval - 5.95 Gy		
	bridges	fragments	DM	bridges	fragments	DM
4	1.03±0.10	-	-	1.31±0.49	1.73±0.27*	0.9±0.13
14	1.01±0.05	-	-	1.29±0.29	1.21±0.31*	0.51±0.15
20	1.00±0.07	-	-	1.17±0.19	0.53±0.07*	0.23±0.06
25	1.01±0.01	-	-	1.11±0.23	0.09±0.01*	0.03±0.003
30	1.02±0.03	-	-	1.03±0.31	-	-

- absence of chromosomal aberrations, * - P <0.05, DM - double minute chromosomes

The table data shows a high level of stability of carpological population of MDBK cells obtained in the culture medium decontaminated by γ-irradiation and subjected to double radiation in adapting (0.05 Gy) and test (5.95 Gy) doses of γ-rays. Based on the above data the irradiated MDBK cell culture can be attributed to the group of stable cells under the karyotype of cell lines.The results of virological studies have shown that the use of culture medium irradiated with γ-rays in a sterilizing dose ($1x10^4$Gy) resulted in an increase in virus titer by 1.10 times (P <0.05) compared with the control, that is more for 9.8% than in the control.

DISCUSSION.

It has been shown that exposure of NBS at doses ranging from 0.1 to $3.0x10^4$Gy does not lead to a substantial increase in the content of toxic products of radiolysis of proteins, carbohydrates and lipids in culture medium, which, in our opinion, should not affect its biological properties. The blood serum irradiation with γ-rays at a dose of 5.0 to $6.0x10^4$Gy caused a significant increase in the formation of radiation induced toxic radiolysis products.The data obtained in the previous series of research and concerning the assessment of physico-chemical, biochemical and toxicological properties of the serums irradiated with γ-rays served as a basis for further researches on studying the effect of irradiation on the proliferative activity of the cell culture, growing them in the irradiated culture media. The results of studying the proliferative activity of MDBK cell cultures grown in the media irradiated with different doses of γ-rays have shown that irradiation of culture media with γ-rays in a dose range of 0.1-$3.0x10^4$Gy has no significant effect on the growth and proliferation of cells that do not contradict to the literature data.Taking into account the literature data that the repeated exposure of cultured cells (lymphocytes) in small (0.1 Gy) doses at the beginning and then - in high doses (5.0 Gy) results not only in a significant increase of survival of the irradiated cells, but in a stimulation of their reproductive capacity, increasing the cell concentration in the culture process [10], we carried out the following series of experiments to study the possibility of stimulating action of γ-rays on MDBK cell culture by repeated irradiation, but in small doses of ionizing radiation. The results of experiments carried out have shown that the maximum accumulation occurs after 48 hours of culturing the doubly irradiated cells in a monolayer at a dose of 0.05 Gy and 5.95 Gy with the interval of 3 min between irradiations, when their concentration is $2.01±0.09x10^6$ cells/cm^3 with a proliferation index of 3.2. The maximum concentration of cells irradiated in suspension at the indicated doses also occurs after 48 hours of culture, constituting $1.99x10^6$ cells/cm^3 with a proliferation index of 2.7.Thus, the pre-exposure of cells in low dose (0.05 Gy) and repeated irradiation at a dose of 5.95 Gy with the interval of 3 minutes, has a stimulating effect on the growth and development of MDBK cell culture, increasing the concentration of cells in 1.77 and 1.70 times (P <0.05) with a proliferation index of 3.2 and 2.7 respectively compared to 2.1 in the control.The results of our cytogenetic studies of MDBK cells irradiated in

a small (0.05 Gy) and large (3.0-10.0 Gy) doses of γ-rays have shown that double (0.05 Gy, 3 min interval - 3.0, 5.0, 7.0 and 10.0 Gy) radiation had much smaller cell chromosome disorders of the studied test culture. For this variant of radiation effect on cells it is characteristic that the tendency to reduce chromosomal aberrations in the form of bridges, fragments and double mini-chromosomes and, the most important, reducing the number of fragments resulting from chromosome rupture leading to apoptotic cell death have been observedboth at small (3.0, 5.0 Gy) and large (7.0; 10.0 Gy) doses of γ-rays. Perhaps the increase in radio resistance of cells of studied culture occurs at repeated irradiation due to the inclusion of reparative processes at achieving a certain level of cell damage.Thus, the irradiation dose of MDBK cells of 0.05 Gy is adapting that leads to a statistically significant (in 2.91 times, $P < 0.01$) decrease in the yield of chromosomal aberrations (bridges, fragments and double minute chromosomes) induced by the testing radiation after repeated exposure to relatively high doses (3.0-5.0 Gy) at the interval of 3 min between the radiogenic effects.Our results suggest that the development of adaptive response in MDBK dividing cells after double exposure in the adapting and test doses leads to an increased efficiency of DNA repair of the chromosomal fragmentation and breaks caused by radiation by reuniting all of its tuft ends in the places of breaks. Perhaps the adaptive response initiation leads mainly to a slower activation (during the long-term cultivation) of stable repair of DNA chromosomal breaks (fragmentations) by a homologous recombination.Since in our experiments we have used both large (sterilizing) and small (stimulating) doses of γ-rays ^{60}Co at the construction of culture medium, the following question is of interest - what is the virus reproducing ability of the transplantable MDBK-02 cell line (fetal bovine kidney cells double irradiated with γ-radiation). The study results showed that the maximum accumulation of IRT virus in control occurs within 48 hours after infection and reaches 6.1±0.11 $lgTCD_{50}/cm^3$ at a multiplicity of infection within 3±0.05 $lgTCD_{50}$/cells. The use of culture medium irradiated with γ-rays in a sterilizing dose ($1x10^4$Gy) for growing the non-irradiated cells caused an increase in virus titer by 1.10 times ($P < 0.050$) compared to the control.

CONCLUSIONS.

1. The double exposure of MDBK cell cultures in small (0.05 Gy) and high (5.95 Gy) dose with the interval of 3 min ensures the development of adaptive cell response to the ionizing radiations, which increases survival to the lethal irradiation (radio resistance development), proliferation index and final cell density.
2. Pre-exposure of cells in the adapting dose of γ-rays prevents the development of processes associated with the induction of genomic instability, accompanied by an increase of chromosomal damages (bridges, fragments, double minute chromosomes).

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