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A simple method of alpha irradiation for experiments in radiobiology

J. Soto [a], C. Sainz [a,*], S. Cos [b], D. Gonzalez Lamuño [c]

[a] *Departamento de Física Médica, Facultad de Medicina, Universidad de Cantabria, E-39011 Santander, Spain*
[b] *Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, E-39011 Santander, Spain*
[c] *Departamento de Genética y Pediatria, Facultad de Medicina, Universidad de Cantabria, E-39011 Santander, Spain*

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Abstract

This paper presents an irradiation system for radiobiology experiments using alpha particles in vitro. Irradiation is delivered to the cells by the presence of radioactive isotopes previously dissolved in the culture medium. The system uses a source of ^{226}Ra from which the radon (^{222}Rn) generated diffuses through the apparatus and is dissolved in a standard culture medium subsequently used for cell culture. The radioactive elements present are the naturally occurring radioactive gas radon and its short-life progeny. The radiation delivered to the cells is mainly composed of alpha particles from ^{222}Rn and its daughters ^{218}Po and ^{214}Po. The doses absorbed by the cell cultures range from 1 to 1000 mGy depending on the conditions of exposure. To illustrate use of the system, we present results obtained when low doses of alpha particles were employed to irradiate CD34+ hematopoietic progenitor cells and MCF-7 human breast cancer cells.

Keywords: Alpha particles; Irradiation system; Radon

1. Introduction

Most studies of the effects of ionizing radiation on cell populations have used X-rays or gamma-rays as the interacting agents. Both X-rays and gamma-rays are easy to handle with different irradiation equipment and deliver directly measurable homogeneous doses to the target. However, irradiation with alpha particles has been less widely used. In contrast to low linear energy transfer (LET) radiation, alpha particles are difficult to use because they undergo attenuation before reaching the target, because the delivered dose may not be homogeneous and because this dose is difficult to measure and must be calculated.

However, in spite of the difficulties with this type of irradiation, there are good reasons to use alpha particles as the interacting agent. These are based on the fact that alpha particles produce effects that are very different, at least in quantitative terms, from those produced by X-rays and

* Corresponding author. Tel.: +34-9422-01974; fax: +34-9422-01903.
E-mail address: sainzc@unican.es (C. Sainz).

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gamma radiation. When the average dose to a cell culture is small, irradiation with alpha particles does not distribute the dose evenly, but rather a small number of cells can receive a large amount of energy. In these conditions, it has been estimated that the dose to a given cell nucleus may be >1 Gy [1], whereas most cells receive no dose at all.

It is perhaps because of these microdose characteristics that alpha particles and other types of high LET radiation produce quantitatively different effects from those of gamma radiation when they interact with the DNA of the cell. Thus, whereas low LET radiation mainly causes damage to the bases or single chain breakpoints, alpha particles more commonly cause breaks in the double chain [2], which are more difficult to repair, with the possibility of repair depending on the dose rate. This characteristic seems to be critical when determining differences in the relative biological effectiveness (RBE) of alpha particles as compared with X-rays or gamma radiation. Especially in the low-dose range, the RBE value attributed to alpha particles is not well known, and depends on the biological response under study [3]. Thus, for the effects due to cell death, the RBE of alpha radiation depends on the cell type and usually shows values below 10 [4]. In contrast, in the induction of chromosomal aberrations in human lymphocytes, values of 18 have been reported [5], and experiments involving the induction of sister chromatid exchanges have demonstrated effects not observed with gamma radiation, suggesting an infinite RBE value [6]. However, the relative effect of alpha particles in comparison with X-rays in the assessment of the risk of late effects [7] remains unclear, even though this value is essential to calculate the equivalent dose per population from the absorbed dose according to different models simulating irradiation to the lung from radon progeny.

All these characteristics of the effects of alpha radiation make it advisable to carry out more experiments with alpha particles as the interacting agent. This paper describes a simple method to irradiate cell cultures with alpha particles, gives the associated dosimetry, and provides some examples of the effects observed in cell populations.

2. Proposed system of irradiation

The systems most commonly used to irradiate cells by alpha particles are based on external particle sources or alpha emitting isotopes introduced into the cell culture medium.

The irradiation systems based on external sources may use radioactive samples emitting alpha particles [8–10] or particle accelerators [11–15]. Both types of system are used to irradiate cells cultured in modified Petri dishes and adhering to the bottom of the dish. Alpha particles from the radioactive sample or from the accelerator pass through the Mylar bottom of the dish, impinge on the cells and deliver the dose to them. In spite of the precision that can be achieved in irradiating a given number of cells or even parts of the cell, these methods are not suitable for cell cultures in suspension.

There are other methods of irradiation in which radioactive elements are introduced into the cell culture medium. The elements most commonly used are those from natural radioactive series, such as ^{212}Bi, obtained by radiochemical separation from ^{224}Ra sources [16,17], or from ^{222}Rn progeny [18]. Since radon is a gas, it is introduced into the culture medium by means of a bubbling device. This method is appropriate for irradiating cells in suspension, but the turbulence created by the bubbles would seem to make it unsuitable for adherent cell lines that grow on the surface of the culture dish.

The system of irradiation proposed here is based on dissolving radioactive isotopes in the culture medium, but these are introduced by diffusion and not by a pumping device. The system consists of a source of ^{226}Ra which is connected directly to the test tubes containing the cell culture medium, as shown in Fig. 1. The source is a solution of $RaCl_2$ in 0.1 N of HCl at a ^{226}Ra concentration of 10.28 μCi/g, with no radioactive impurities other than the progeny. The solution is contained in a 10 ml capsule surrounded by a lead jacket. From the capsule comes a plastic tube and the radon generated diffuses along this tube to the other end, which is attached to a 50 ml container with the culture medium. The end of the tube has a cover that is specially adapted to the container to

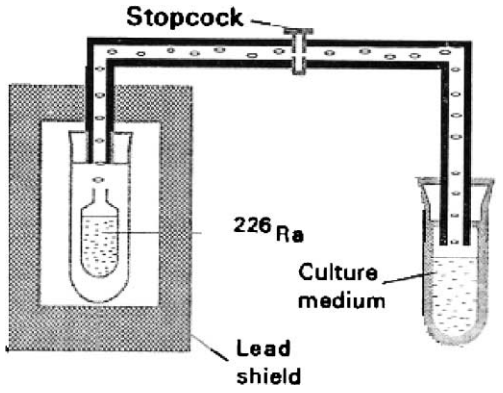

Fig. 1. Experimental apparatus to dissolve radon in the cell culture medium.

ensure that the joint is leakproof, thereby preventing loss of the gas as it diffuses from the source to the culture medium. In addition, the connecting tube has a stopcock that cuts off the flow of radon when not in use.

The gas is dissolved in the medium to be irradiated when the radon generated by the source diffuses and comes into contact with the medium. Once in contact, the radon tends to dissolve in the medium until saturation point is reached, when the difference in partial pressures between the gas in the air and that in the medium prevents further passage of the gas from one phase to the other.

When the culture medium has been exposed for a certain time, the tube is closed and shaken, and the amount of dissolved radon is measured by means of gamma spectrometry equipment. Measurement is performed after 3 h, when radioactive equilibrium has been reached between radon and its short-life progeny. The equipment consists of a GeHp (Canberra) semiconductor detector protected by a lead casing, linked to to a "cold finger" cooled with liquid nitrogen and connected to a multichannel analyzer. A calibrated sample with ^{226}Ra of known activity and similar geometry to that of the containers with the irradiated medium is used, and the radon activity of the culture medium is calculated from the counts recorded at photopeaks of 352 keV (^{214}Pb) and 612 keV (^{214}Bi). It can be assured that the measured activity represents the real activity dissolved in the culture medium, because the air volume inside the tube above it is negligible.

The final radon concentration in the medium depends on the time of exposure to the source and the flow rate of the gas through the connecting tube (practically constant in the conditions used in the study). The typical concentrations used for experiments in the 10^5–10^6 Bq/l range are reached after an exposure time of 2 h. The maximum concentration of radon dissolved in the medium with this apparatus (i.e. to saturation point) is 10^7 Bq/l, which is achieved after 24 h exposure to the source.

The system offers certain advantages over other systems that are commonly used in radiobiology experiments. The most important are its simplicity and its versatility, since it can be used to irradiate both cell cultures in suspension and those growing on the surface of Petri dishes. In addition, the initial shake of the tube and the waiting times before measurement of activity and subsequent cell culture allow homogeneous distribution of the emitters in the medium.

3. Dosimetry

With the proposed irradiation system, absorbed doses are due to alpha emissions from ^{222}Rn and its daughters ^{218}Po and ^{214}Po. The energy of the alpha particles emitted is 5.49, 6 and 7.68 MeV, respectively. In aqueous medium, the range of these particles is 42.8 μm for ^{222}Rn, 49.5 μm for ^{218}Po and 74.2 μm for ^{214}Po. The energy deposited by beta and gamma emissions and the probability of alpha emission from other progeny are so low that their contribution to the total dose is insignificant.

For a given radon concentration in the medium, the absorbed dose for an experiment in vitro depends on the conditions in which the culture is performed. As a gas, radon tends to escape from the medium in which it is dissolved into the atmosphere, where its partial pressure is lower. For this reason, in experiments where cells are cultured in open dishes, the radon will escape into the air without contributing to the dose absorbed by the culture. In contrast, if cells are cultured in closed tubes, the radon will be present for the whole experiment and will contribute to the irradiation. To

illustrate these two possibilities, we present the results obtained by irradiating two cell lines cultured in different conditions.

3.1. Anaerobic experiments

In this type of experiment, the culture with the irradiated medium remains hermetically sealed throughout, and both the cells and growth factors are injected into the tube containing the medium with radon in equilibrium with its short-life progeny. Thus, the alpha radiation is due to emissions from the ^{222}Rn, ^{218}Po and ^{214}Po isotopes dissolved in the medium.

The evolution over time of the absorbed dose during the experiment is given by

$$D(t) = (E_\alpha(^{222}\mathrm{Rn})\lambda_{^{222}\mathrm{Rn}}N_{^{222}\mathrm{Rn}}(t) + E_\alpha(^{218}\mathrm{Po})\lambda_{^{218}\mathrm{Po}}N_{^{218}\mathrm{Po}}(t) + E_\alpha(^{214}\mathrm{Po})\lambda_{^{214}\mathrm{Po}}N_{^{214}\mathrm{Po}}(t))/m, \quad (1)$$

where E is the energy of the alpha emission for each isotope; λ_i, the disintegration constants; N_i, the populations at time t for each isotope; m, the mass of the irradiated sample (culture medium plus cells).

The total absorbed dose in an experiment can be calculated by integrating this expression into its duration. It is, therefore, necessary to calculate the instantaneous populations for each alpha emitter, by solving the Bateman equations,

$$\begin{aligned} \frac{\mathrm{d}N_0}{\mathrm{d}t} &= -\lambda_0 N_0, \\ \frac{\mathrm{d}N_1}{\mathrm{d}t} &= \lambda_0 N_0 - \lambda_1 N_1, \\ \frac{\mathrm{d}N_2}{\mathrm{d}t} &= \lambda_1 N_1 - \lambda_2 N_2, \\ \frac{\mathrm{d}N_3}{\mathrm{d}t} &= \lambda_2 N_2 - \lambda_3 N_3. \end{aligned} \quad (2)$$

(0: ^{222}Rn, 1: ^{218}Po, 2: ^{214}Pb, 3: ^{214}Bi (in equilibrium with ^{214}Po)).

This can be solved numerically with an iterative calculation program, and bearing in mind that initially radioactive equilibrium exists between radon and its progeny, it can be shown that

$$\lambda_0 N_0(0) = \lambda_1 N_1(0) = \lambda_2 N_2(0) = \lambda_3 N_3(0). \quad (3)$$

Since the half-life of ^{222}Rn is considerably longer than the irradiation time, the dose rate remains practically constant throughout the experiment. In addition, the concentration of alpha emitters dissolved in the culture medium hardly changes, so that the absorbed dose during the experiment is proportional both to this equilibrium concentration and to the length of the experiment.

As an example of this type of irradiation, experiments were performed on CD34+ hematopoietic progenitor cells. The effect of low doses (0.3 and 0.6 Gy) of alpha radiation due to radon and its progeny on cell growth, early apoptosis and total cell death (late apoptosis and/or necrosis). Also analysed was the response to different times of exposure to the irradiated medium.

Both the radon-containing medium and the control medium were hermetically sealed in 5-ml glass tubes and the tubes kept sealed throughout the experiment. After determination of the amount of radon in the culture medium, the cells, together with growth factors (cytokines), were injected into the tubes and analysed after different exposure times.

The experiment was performed for different irradiation times and cultures. The initial cell population was divided into two parts. The first was irradiated for 24 h, for a total absorbed dose of 0.3 Gy; the second, for 48 h and a total dose of 0.6 Gy.

The results are shown in Fig. 2. In comparison with controls, cultures in the irradiated medium presented increased cell growth, and significant reductions in both early apoptosis and total cell death. The greatest differences were observed for cultures irradiated for 48 h.

3.2. Aerobic experiments

In this case, the cultures were kept in open dishes throughout the experiment. The radon initially dissolved in the medium diffuses out, leaving only its short-life progeny, ^{218}Po and ^{214}Po, as the source of irradiation.

To confirm the release of radon into the air from the medium in the open dishes, the following experiment was carried out. A certain quantity of radon was dissolved in the standard culture medium and, when radioactive equilibrium was

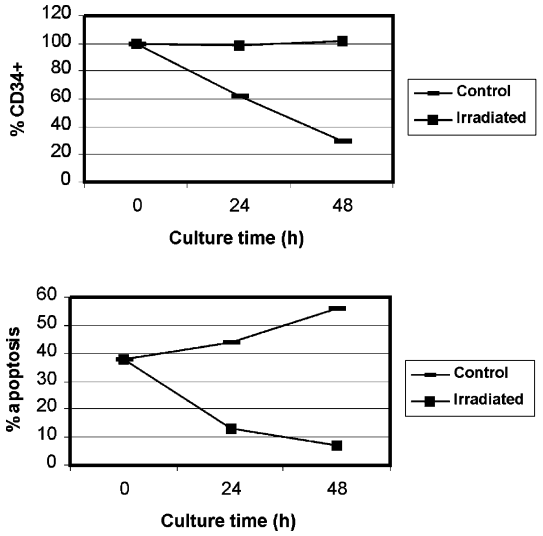

Fig. 2. Survival and early apoptosis after irradiation for 24 and 48 h in CD34+ cells.

reached, the concentration was determined. Then, 5 ml of medium was removed and placed in a Petri dish and gamma spectrometry was used to measure the population of ^{214}Bi at 3-min intervals.

The initial concentration of radon in equilibrium was used and the Bateman equations were solved under the assumption that the gas escapes. Solving equations for 3-min intervals yields theoretical ^{214}Pb and ^{214}Bi populations for the measurement times, provided that there is no radon in the medium.

Fig. 3 compares the counts obtained for ^{214}Bi and the calculated theoretical values. The comparison confirms the hypothesis that no radon is present in the medium and, therefore, makes no contribution to the dose absorbed by the culture.

Proceeding analogously with the solution of Eqs. (1)–(3), we find that the dose is proportional to the initial concentration of radon dissolved in the culture medium.

For aerobic irradiation, a second type of experiment was performed to study the influence of radiation on MCF-7 human metastatic breast cancer cells combined with the chemotherapeutic agents taxol and VP-16. In this case, cells were cultured in open dishes so that most of the initially dissolved radon escaped from the medium into the atmosphere.

In these circumstances and owing to the short half-life of the isotopes present in the culture, the dose rate decreases considerably over the experiment, as can be seen in Fig. 4.

The doses received by the cells were lower than in the anaerobic experiments (sealed tubes) and ranged between 2 and 15 mGy. These doses were calculated from the initial concentration of radon in equilibrium with its daughters. In this calculation, it is assumed that all the dissolved radon is lost during filtration of the medium and subsequent exposure of the open dishes to the atmosphere.

The results indicate that the alpha radiation at the doses used enhances the effect of taxol [19], and to a lesser extent that of VP-16, on cell survival and viability; these variables were significantly reduced in comparison with controls.

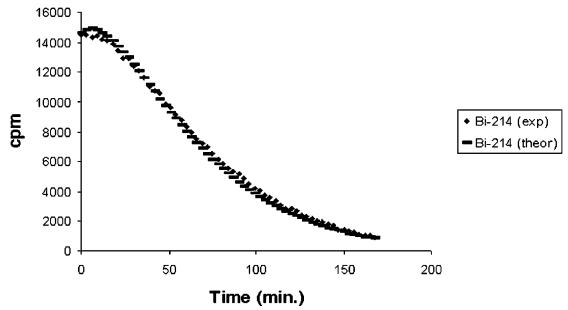

Fig. 3. Comparison of theoretical and experimental values for ^{214}Bi made to confirm the hypothesis that no radon is present in the medium in aerobic experiments.

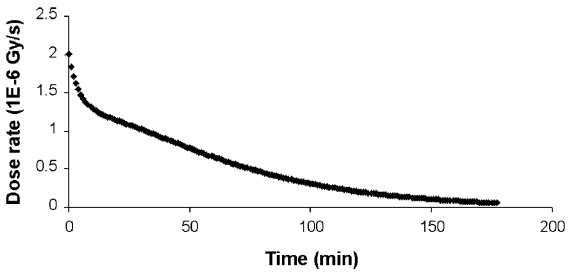

Fig. 4. Evolution of the dose rate in aerobic experiments (dose rate versus time).

4. Discussion

The method presented here makes it possible to obtain different concentrations of dissolved radon in the culture medium employed in low-dose experiments in vitro and gives an even distribution of the emitters in the medium. In comparison with other systems that are widely used in radiobiology, we would emphasise its simplicity and the possiblity of irradiating both cell cultures in suspension and those growing on the surface of culture dishes. In addition, irradiation with this method resembles conditions in vivo more closely than that provided by other systems.

The irradiation produced is due to alpha particles from ^{222}Rn and its daughters ^{218}Po and ^{214}Po, or due only to the progeny if cells are cultured in open dishes. These two possibilities were illustrated by the results of irradiating cell lines cultured in different conditions. CD34+ hematopoietic progenitor cells cultured in sealed tubes were used to evaluate the effect of low doses (0.3–0.6 Gy) of alpha particles on survival, apoptosis and total cell death, and significant differences were found between cells irradiated with radon and untreated cells. In contrast, MCF-7 metastatic human breast cancer cells were cultured in open dishes and analysed to determine the combined effect on survival, viability and apoptosis of very low (2–15 mGy) alpha particle doses plus the cytotoxic agents Taxol and VP-16. The results again showed significant differences between irradiated cultures and controls.

The use of alpha particles is of interest because of their effects, some of which are not observed with low LET radiation like X-rays or gamma-rays. The main problem with this type of irradiation lies in the uneven deposition of energy in the irradiated medium. This is because the dimensions of the targets (cells or cell nuclei) are of the same magnitude as the range of the particles. Particularly at low concentrations, the distribution of the dose in the culture is not homogeneous. For this reason, analysis of the cell response to alpha particles involves the use of microdosimetry methods [20].

To estimate the percentage of cells affected by an alpha particle in the two types of experiment, we performed a Monte Carlo simulation. The variables in the simulation were the cell radius and the concentration of emitters in the sample. For the anaerobic experiments (emitters and cells in suspension) with typical concentrations of 10^6 Bq/l, the percentage of affected cells was found to be about 5% (with cell radius varying between 4 and 6 μm). In the aerobic experiments, the cells were attached to the bottom of the culture dish, with the emitters in suspension. Owing to the absence of radon, the percentage of directly affected cells was lower than in the anaerobic experiments and was about 0.1%.

These low percentages of direct interaction, together with the observation of the effects on the whole culture, indicate the existence of lesions other than direct damage to the DNA, that is, the existence of extranuclear targets [21]. The extension of the effect to unirradiated cells, the so-called "bystander effect" [22], has been explained by several mechanisms. One is the generation of superoxide anions [23]. Hydrolysis of water or breakdown of other molecular components in the medium generates highly oxidative free radicals capable of penetrating a cell and affecting its internal structure, including its DNA. Another mechanism that has been proposed is the release by the affected cells of cytokines and other extracellular factors [24] capable of raising intracellular levels of superoxide anions in unirradiated cells. Finally, an explanation has also been put forward based on the transmission of the effect produced on a few cells to others by way of the gap junctions or intercellular connections [25,26]. However, this explanation is not valid for the examples presented here, since in tumour cells these connections lose their function, as also occurs in culture in vitro of cells in suspension.

References

[1] UNSCEAR 2000 Report to the General Assembly with Annexes, New York, Volume II: Effects, United Nations Publication, Sales No. E.00.IX.4, New York, 2000.
[2] J.F. Ward, Radiat. Res. 142 (1995) 362.
[3] K. Baverstock, M. Thorne, Int. J. Radiat. Biol. 74 (6) (1998) 799.
[4] International Commission on Radiological Protection, Annals of the ICRP.20(4) Pergamon Press, Oxford, 1989.

[5] A. Edwards, R. Purrott, J. Prosser, D. Lloyd, Int. J. Radiat. Biol. 38 (1980) 83.
[6] S. Aghamohammadi, D. Goodhead, J. Savage, Int. J. Radiat. Biol. 53 (1988) 909.
[7] H. Engels, H.G. Menzel, P. Pihet, A. Wambersie, Strahlenther Onkol. 175 (Suppl. 2) (1999) 47.
[8] N.F. Johnson, T.R. Carpenter, R.J. Jaramillo, T. Liberati, Environ. Health Perspect. 105 (Suppl. 4) (1997).
[9] M. Kadhim, D. Macdonald, D. Goodhead, S. Lorimore, S. Marsden, E. Wright, Nature 355 (1992) 738.
[10] S. Lorimore, D. Goodhead, E. Wright, Int. J. Radiat. Biol. 63 (5) (1993) 655.
[11] C. Soyland, S. Harsfield, H. Steen, Radiat. Res. 153 (1) (2000) 9.
[12] J. Nelson, A. Brooks, N. Metting, M. Khan, R. Buschbom, A. Duncan, R. Miick, L. Braby, Radiat. Res. 145 (1996) 568.
[13] T. Hei, L. Wu, S. Liu, D. Vannais, C. Waldren, G. Randers-Pehrson, Proc. Natl. Acad. Sci. USA 94 (8) (1997) 3765.
[14] R. Miller, M. Richards, D. Brenner, E. Hall, R. Jostes, T. Hui, A. Brooks, Radiat. Res. 146 (1) (1996) 75.
[15] L. Wu, G. Randers-Pehrson, A. Xu, C. Waldren, C. Geard, Z. Yu, T. Hei, Proc. Natl. Acad. Sci. USA 96 (1999) 4959.
[16] R. Atcher, J. Freidman, J. Hines, Appl. Radiat. Isot. 39 (1988) 283.
[17] N. Metting et al., Radiat. Res. 132 (1992) 339.
[18] R.F. Jostes, Radiat. Res. 127 (1991) 211.
[19] J. Soto, C. Sáinz, D. Gonzalez-Lamuño, S. Cos, Oncol. Rep. 7 (2000) 941.
[20] E. Polig, Curr. Top. Radiat. Res. 13 (1978) 189.
[21] A. Deshpande, E. Goodwin, S. Bailey, B. Marrone, B. Lehnert, Radiat. Res. 145 (1996) 260.
[22] A.J. Grosovski, Prot. Natl. Acad. Sci. USA 96 (1999) 5346.
[23] P. Narayanan, E.H. Goodwin, B. Lenhert, Cancer Res. 57 (1997) 3963.
[24] B. Lehnert, E. Goodwin, Cancer Res. 57 (1997) 2164.
[25] L. Yang, Y. Chiang, H. Lenz, K. Danenberg, C. Spears, E. Gordon, W. Anderson, D. Parekh, Hum. Gene Ther. 20 (1998) 719.
[26] E. Azzam, S. de Toledo, T. Gooding, J. Little, Radiat. Res. 150 (1998) 497.