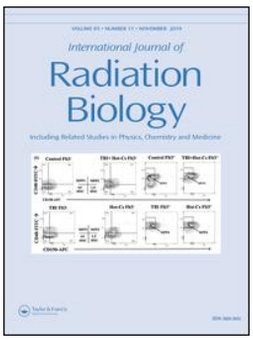

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ORIGINAL ARTICLE

Isolation of the effects of alpha-related components from total effects of radium at low doses

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ABSTRACT

Purpose: Radium is the most common source of alpha radiation exposure to humans and non-human species in the environment but the dosimetry is complicated by the decay chain which involves gamma exposure due to radon daughters. This paper seeks to determine the separate contributions of alpha and gamma doses to the total dose and total direct and non-targeted effect in a fish and a human cell line.

Materials and methods: This study aimed to isolate the effect of alpha particles following exposure to low doses of radium in cells, and their progeny which received no further exposure. This was initially done by comparing the survival values of a human keratinocyte cell line (HaCaT) and an embryonic Chinook salmon cell line (CHSE-214) exposed to gamma radiation, from survival of the same cell lines exposed to mixed alpha and gamma radiation through exposure to Ra-226 and its decay products. A Monte Carlo simulation was later performed to determine the contributions of radium decay products including radon daughters.

Results: The human cell line showed increased radioresistance when exposed to low doses of alpha particles. In contrast the fish cell line, which demonstrated radioresistance to low dose gamma radiation, showed increased lethality when exposed to low doses of alpha particles. Significant and complex levels of non-targeted effects were induced in progeny of irradiated cells. The simulation showed that gamma and beta decay products did not contribute significant dose and the highest beta dose was below the threshold for inducing non-targeted effects.

Conclusions: The results confirm the need to consider the dose-response relationship when developing radiation weighting factors for low dose exposures, as well as the need to be aware of possible cell line and species differences.

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Introduction

In dosimetry, linear energy transfer (LET) is the amount of energy (keV) that an ionizing particle transfers to the material transversed per unit distance (μm). It describes the quality of different types of radiation. It describes the action of radiation into matter. Photons such as gamma rays are able to traverse great distances unchanged before being entirely absorbed, however monoenergetic ions such as alpha particles cause frequent direct ionizations within a smaller range. Due to this, exposures to alpha particles are often considered as producing a significantly higher concentration of damage given the same amount of absorbed energy when compared to gamma radiation (Goodhead 1994). The derivation of concepts such as relative biological effectiveness (RBE) and the assignment of weighting factors have further supported this notion, aligning dose with biological effect in one seamless system. In vitro studies of alpha radiation effects have returned a wide range of RBE values, from <2 for the induction of double strand breaks, to 3.5–10 for cell lethality and transformation in different cell lines, to >25 for other endpoints assessed (Thomas et al. 2007). Most countries accept the single recommended radiation weighting factor of 20 for alpha particles when specifying exposure limits (ICRP 2007). A simplified relationship between dose and effect is useful in developing an all-encompassing regulatory policy for protecting against radioactive exposure, however, not all cases are alike. While existing RBE values and weighting factors may be true for high doses, there are many laboratory studies casting doubt that such relationships hold after exposure to low doses and low dose rates. This is particularly relevant in situations with risks of prolonged low dose exposure, including the mining and milling of uranium for nuclear power plants, and the handling of nuclear waste.

It has been shown that if a single alpha particle traverses a cell, it causes a significantly lower risk of oncogenic transformation than expected (Miller et al. 1999). Further, the work of Nagasawa and Little has shown significantly higher frequencies of mutation than would be expected through linear extrapolation from data for high doses, at doses where the *mean* number of alpha particle traversals per nucleus was significantly less than one, i.e. many cells in the flask

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got no traversal (Nagasawa and Little 1999). At low doses, both alpha and gamma rays can cause non-targeted effects (NTE) like genomic instability where damage does not cause direct mortality and cells appear completely normal but de novo con-clonal effects are seen in distant progeny and lethality occurs generations later, characterized by a reduction in plating efficiency or biochemical changes in the progeny of irradiated cells (Seymour et al. 1986). Cell survival at sub-lethal doses of gamma irradiation has also been observed to differ from what is expected by the traditional linear-quadratic model, instead displaying a region of low-dose hyper-radiosensitivity (HRS) followed by increased radioresistance (IRR) in both cells surviving exposure to ionizing radiation and the progeny of these cells (Marples et al. 1997).

Of particular interest to this study is whether NTE amplify low dose effects such that they are higher than what would be expected from the established linear no-threshold model (LNT) following exposure to low doses of an environmental alpha emitter: radium-226. Average natural radium-226 concentration in soil can vary from 3.7 to 126 Bq/kg around the world, however areas of high natural background can present concentrations up to three orders of magnitude higher than average. Natural activities in surface water generally range between 0.5 and 20 mBq/L, rising to 3000 mBq/L surrounding mining/milling sites. Further, monitoring data following human activities such as hydro-fracking have observed values up to 620 Bq/L of radium-226 (Fesenko et al. 2014). Despite being an alpha emitter by itself, it is known that the uranium decay chain of which radium is part of involves many gamma and beta emissions, thereby making it difficult to measure pure alpha effects. To approach this problem two methods were considered. In the first approach survival curve data generated by our group for gamma irradiated cells exposed to acute Cs-137 or chronic Ra-226 were analyzed. This study measured survival and the lethal mutation phenotype assayed as reduced cloning efficiency in cultures of a human keratinocyte cell line (HaCaT). In addition, due to the increasing relevance of protecting non-human biota from radium in hydrogeologic contaminations from mining, etc., the study also investigated relative alpha exposure effects in the embryonic Chinook salmon cell line (CHSE-214) because to the importance of radium exposure in aquatic ecosystems left behind after uranium mining activities .

In the analysis data from the gamma irradiation experiments (through acute exposure to Cs-137) were subtracted from data generated from experiments with mixed alpha, beta and gamma irradiation (through chronic exposure to Ra-226 and its progeny). The data were analyzed to see the actual effect of caesium-137 exposure in directly exposed and distant progeny versus chronic exposure to radium assuming a dose and dose rate effectiveness factor (DDREF) value of 1 (Rühm et al 2016; Hoel 2018). In the second approach a Monte Carlo model was used to determine the contributions of alpha, beta and gamma decays to the total effect of the radium exposure in the directly exposed and progeny cells.

Materials and methods

Cell culture

The HaCaT cell line used in the study is an immortalized human keratinocyte cell line originally derived and characterized by Boukamp et al (Boukamp et al. 1988). The cell line used in this study was obtained as a gift from Dr. Orla Howe (Dublin, Ireland). The cell line was routinely maintained with RPMI-1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Burlington, Canada), 5 ml of 200 mM L-Glutamine (Gibco, Burlington, Canada), 0.5 g/ml hydrocortisone (Sigma-Aldrich, Oakville, Canada), 25 mM Hepes buffer (Gibco), 10 U/ml penicillin (Gibco) and 10 mg/ml streptomycin sulfate, (Gibco). These cells were grown at 37 °C in an incubator with 5% CO_2.

The CHSE-214 is an embryonic cell line derived from Chinook salmon obtained as a gift from Dr. Neils Bols (Waterloo, Canada). CHSE-214 cells were cultured in Leibovitz's L-15 medium supplemented with 12% fetal bovine serum (Invitrogen), 5 ml of 200 mM L-Glutamine (Gibco), 25 mM Hepes buffer (Gibco), 10 U/ml penicillin (Gibco) and 10 mg/ml streptomycin sulfate (Gibco). These cells were grown at 19 °C in an incubator without CO2.

Reduction in cloning efficiency was observed using the clonogenic assay technique developed by Puck and Marcus (Puck and Marcus 1955). Cell stocks were maintained in T75 flasks with 30 ml medium. For clonogenic assay the cells were seeded into 4 replicate T25 flasks with 5 ml fresh culture media at the required cell density such that at least 100 viable colonies could be expected to form in control flasks.

Reporter T25 flasks were maintained in the incubator for 9 days (HaCaT cells) or 40 days (CHSE-214 cells). Following the incubation period, colonies in sham irradiated (control) flasks were visible to the naked eye. Flasks were stained using a 1:4 (v/v) dilution of Fuchsin-Carbol (Ricca Chemical Co., Arlington, TX) in water, and macroscopically visible colonies (confirmed to have more than 50 cells when observed under a microscope) were scored as survivors. The forth flask in each group was grown to confluence and sub-cultured to determine progeny 2 and 3 survival using the method in Seymour et al (1986).

Chronic irradiation using Ra-226 in medium

Stock solutions of medium containing the radioisotope ^{226}Ra were prepared using neutralized radium nitrate (Eckert & Ziegler 2010, Valencia, USA). 100 ml L-15 or RPMI medium was mixed with 1000 Bq of ^{226}Ra solution. The concentration of ^{226}Ra in this stock medium was 10,000 mBq/ml. After filtering into storage tubes, serial dilutions were made to give the required final concentrations.

500 cells were initially seeded into T25 flasks containing 5 ml of medium with Ra-226 or control medium. 4 flasks were prepared for each respective concentration: 0, 0.1, 1, 10, 100, 200 or 500 Bq/ml Ra-226. Flasks were maintained in the incubator for 9 days after which the radioactive medium was removed from both cell lines, and the cells were gently rinsed with calcium and magnesium-free DPBS. Ra-226

residues in the flasks were assumed to be insignificant. Flasks then received 5 ml of fresh culture medium without Ra-226 and returned to the incubator. 3 flasks from each concentration were deemed reporter flasks, incubated for 9 or 40 days, and stained as described above. Cloning efficiencies observed in these reporter flasks represented the initial plating efficiencies from direct chronic irradiation. The remaining fourth flask of each concentration was left to incubate until 80–90% confluency, after which it was subcultured as described above seeding 500 cells into a fresh flask. From here on however no further irradiation was done and all flasks received fresh culture medium containing 0 Bq/ml Ra-226. The process was repeated as before, and cloning efficiencies observed in these reporter flasks represented survival fractions of the progeny (P2). The process was repeated once more to observe further change in the cloning efficiency in subsequent generations (P3).

Acute irradiation using a Cs-137 source

As with the ^{226}Ra experiments, 4 T25 flasks were seeded with 500 cells for each respective dose: 0, 0.05, 0.1, 0.25, 0.5, 0.75 or 1 Gy. The flasks were incubated for six hours to allow for cells to adhere to the flask, after which they were exposed to their respective γ-ray dose using a ^{137}Cs source (Taylor source, McMaster University, Hamilton, Canada). Flasks were placed at 26 cm from the radiation source, irradiated at a dose rate of 0.273 Gy/min and the room temperature was around 26 °C.

All flasks were placed back in the incubator immediately after irradiation. Similar to the ^{226}Ra experiments, 3 flasks were deemed reporter flasks and incubated for approximately 9 days before being stained as described above (initial). The remaining fourth flask of each dose was incubated until cells became 80–90% confluent, after which they were subcultured as described above with fresh culture medium. This process was also repeated twice as above (P2 and P3).

Determining γ and electron absorbed doses from ^{226}Ra and its daughters

To compute the γ and electron absorbed doses from ^{226}Ra and its daughters, Monte Carlo simulations were carried out using the MCNP6 code. As a simplified geometry for the t-25 flask, a 1 mm thick plastic layer of $10 \times 5\,cm^2$ was used in MCNP. A culture medium was represented as a 3 mm thick layer on top of the plastic layer. The cell layer was assumed to be a 0.1 mm thick sub-layer within the culture medium, on contact with the plastic layer. The air volume of the flask was represented by a 2.2 cm thick layer on top of the culture medium.

As the source particles from the ^{226}Ra decay, two source cards, one for conversion electrons and the other for X-ray and gamma photon. The X-ray and gamma photon energies and emission probabilities were retrieved from Table of Radioactive Isotopes.

(WWW Table of Radioactive Isotopes, http://nucleardata.nuclear.lu.se/toi/) and the conversion electron data were retrieved from Browne et al (1986). The positions of the conversion electrons and the photons were uniformly sampled within the culture medium. Figure 1 shows an example of the source position sampling.

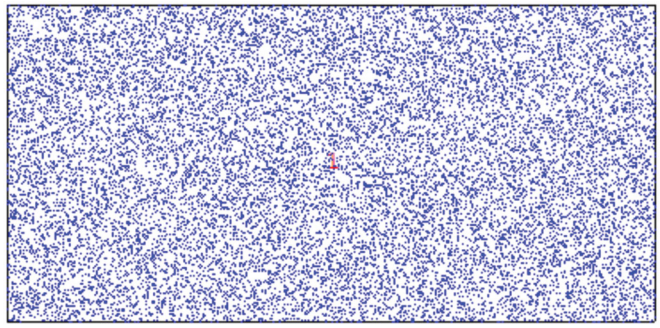

Figure 1. An example of the MCNP source position sampling for the X-ray and gamma photon on the horizontal plane. A culture medium was represented as a 3 mm thick layer on top of a plastic layer ($10 \times 5\,cm^2$, 1 mm thick). The position of each photon source particle was uniformly sampled within the culture medium.

From the list daughter nuclides, beta decay electrons from 214Pb and 214 Bi can make significant contribution to the absorbed dose and therefore, two source cards were created for the beta decay electrons and the conversion electron data were retrieved from Browne et al.'s data table (Browne et al. 1986). Unlike the conversion electron and photon sources, the positions of the beta decay electrons were uniformly sampled from the entire flask volume of $10 \times 5 \times 2.5\,cm^3$, given a large fraction of 222Rn is in the air region of the flask volume.

For each type of source card, the absorbed dose was computed using both F6 and *F8 tallies for either the primary or the secondary electrons, depending on the source card. The absorbed dose values from both tallies were in excellent agreement for each source card.

Table 1 compares the MCNP simulation result for each source card. From the 226Ra decay, the absorbed dose by conversion electrons are eighteen times higher than the dose from the X-ray and gamma photons, which is obvious since electrons always deposit energies to the cell layer as long as their tracks pass through the cell volume while the thickness of the cell layer is much thinner than the mean free path of the most photon energies. For the 214Pb and 214 Bi beta electrons, 214 Bi beta dose is about six times higher than the 214Pb dose, which may stem from the fact that the mean beta energy of 214 Bi is higher, hence a larger percentage of 214 Bi beta particles can penetrate the cell medium and arrive to the cell volume. Between the 226Ra dose and the daughters' beta dose, the daughters' dose rate is about 70 times higher than the 226Ra dose rate under the equilibrium condition. Therefore, the daughters' beta dose is the main low LET dose contributor in any case the exposure time is longer than 10 percent of the half-life of ^{222}Rn.

To compute the absorbed dose for an experimental set using the table MCNP simulation result, the total number of each decay must be computed. For ^{226}Ra decay, its computation is straightforward from the ^{226}Ra activity and exposure time while the integral of the time dependent activity must

Table 1. Absorbed dose computed by MCNP for each type of the source card.

226Ra α decay			
Conversion electron	X and gamma	214Pb β^- decay electrons	214Bi β^- decay electrons
2.36×10^{-14} Gy/decay	1.27×10^{-15} Gy/decay	2.51×10^{-13} Gy/decay	1.47×10^{-12} Gy/decay

Table 2. Survival of HaCaT cells exposed to Cs-137 (2a and Ra-226 (2 b) expressed as percentage surviving fraction ± SD after acute exposure to Cs-137 at a dose rate of 0.273 Gy/min or after chronic exposure to radium-226 for 9 days.

(a) Survival fraction (%) HaCaT exposed to ^{137}Cs							
Dose (Gy)	0	0.05	0.1	0.25	0.5	0.75	1
P0	100 ± 2	93 ± 4	90 ± 3	92 ± 6	86 ± 3	76 ± 2	68 ± 8
P1	100 ± 3	79 ± 7	75 ± 4	85 ± 7	83 ± 7	78 ± 4	92 ± 4
P2	100 ± 3	77 ± 8	79 ± 3	90 ± 3	94 ± 6	96 ± 5	97 ± 9
(b) Survival fraction (%) HaCaT exposed to ^{226}Ra							
Ra-226 (Bq/ml)	0	0.01	1	10	100	200	500
P0	100 ± 1.5	109 ± 3.0	107 ± 3.8	107 ± 3.0	104 ± 2.7	107 ± 3.3	92 ± 1.4
P1	100 ± 2.6	108 ± 5.4	109 ± 8.2	107 ± 3.4	99 ± 6.0	106 ± 4.4	84 ± 3.0
P2	100 ± 2.1	101 ± 2.5	106 ± 3.3	99 ± 2.0	102 ± 2.7	95 ± 2.5	101 ± 2.4
P3	100 ± 2.8	99 ± 2.8	101 ± 3.6	105 ± 4.3	106 ± 3.5	105 ± 1.7	102 ± 2.7

P0 refers to the directly exposed cells and P1, 2 and 3 refer to the passage numbers at which the lethal mutation burden was measured in progeny without further exposure to radiation.

be done in the initial activity buildup phase for ^{214}Pb and ^{214}Bi.

Determination of surviving fractions and progeny survivals

Survival fractions/plating efficiencies of cells are determined as cloning efficiency observed through staining, which visualizes the fraction of colonies formed from the 500 cells plated. Progeny survival fractions were calculated at each observed interval and for each dose through following the recorded cell numbers at the start and end of each interval, in accordance to previous delayed lethal effect assays by (Seymour et al. 1986; O'Reilly et al. 1994; Lyng et al. 1996; Mothersill et al. 2000). Here, the product of the cells observed at the end of the current passage, with the total cell number at the end of the preceding passage, was divided by the initial number of cells seeded per passage corrected for plating efficiency.

To isolate the effect of α-particles on the survival and genomic instability of cells, this study examined the effects observed after γ irradiation (through acute exposure to ^{137}Cs) and from mixed α,β and γ irradiation (through chronic exposure to 226Ra).

Results

Human keratinocyte cell line (HaCaT)

The data obtained for the initial and progeny survival fractions for HaCaT cells exposed to ^{226}Ra and ^{137}Cs are shown in Table 2. Data for acute ^{137}Cs exposure show a marked hypersensitivity in the low dose region followed by a region of increased radioresistance (IRR). The progeny of these cells continue to demonstrate such HRS/IRR behavior with further decrease in cloning efficiency thereby observing lethal mutations in those generations. In particular significant decreases in cloning efficiencies are observed in the first observation of progeny of cells (P2, 16 population doublings) irradiated at 0.05 Gy by 19% ($p = .007$), 0.1 Gy by 23% ($p = .00007$), 0.25 Gy by 14% ($p = .02$), 0.5 Gy by 15% ($p = .01$) and 0.75 Gy by 17% ($p = .0004$). Further significant decreases are observed in the second observation of progeny (P3, approximately 25 population doublings) at 0.05 Gy by 17% ($p = .01$) and at 0.1 Gy by 14% ($p = .001$).

In comparison, progenitor cells exposed to ^{226}Ra show significantly greater survival with many observations of higher cloning efficiency compared to sham irradiated control flasks (denoted as survival values greater than 100% i.e. more colonies survived in the treated flasks compared to controls). As such no HRS region is observed, with little to no change in survival compared to control in cells exposed to concentrations greater than 0.1 Bq/ml of Ra-226. At P2, survival of progeny in concentrations up to 10 Bq/ml of Ra-226 show significantly higher survival compared to control, while observations at P3 show similar survival values to what was observed in the progenitors

Embryonic chinook salmon cell line (CHSE-214)

In contrast to the studied human cell line, there was no significant cell death observed in the directly exposed cells of the CHSE-214 fish cell line and their progeny to acute Cs-137 exposure over the dose range tested (Table 3). This indicates low dose radioresistance when compared to human cell culture and confirms previous data from this group (Ryan et al. 2008). When CHSE-214 cells were exposed to ^{226}Ra however, progenitor cells show a marked response with decreasing cell survival following an almost linear trend with respect to dose. Residual survival observed at P2 (8 doubling periods after removal of radium) show increased lethal mutation however residual survival observed in the subsequent progeny at P3 (16 doubling periods after removal of radium) demonstrate a return of radioresistance with survival values similar to initial values.

Table 3. Survival of CHSE-214 cells exposed to Cs-137 (3a) and Ra-226 (3 b) expressed as percentage surviving fraction ± SD after acute exposure to Cs-137 at a dose rate of 0.273 Gy/min or after chronic exposure to radium-226 for 28 days.

(a) Survival fraction (%) CHSE-214 exposed to ^{137}Cs							
Dose (Gy)	0	0.05	0.1	0.25	0.5	0.75	1
P0	100 ± 3	100 ± 2	101 ± 4	104 ± 3	100 ± 5	125 ± 3	99 ± 3
P1	100 ± 4	97 ± 3	105 ± 6	114 ± 4	118 ± 4	160 ± 6	119 ± 5
P2	100 ± 3	93 ± 2	111 ± 4	113 ± 4	140 ± 5	160 ± 5	139 ± 4
(b) Survival fraction (%) CHSE-214 exposed to ^{226}Ra							
Ra-226 (Bq/ml)	0	0.1	1	10	100	200	500
P0	100 ± 5	69 ± 8	71 ± 13	70 ± 7	55 ± 6	31 ± 6	<0.2
P1	100 ± 16	72 ± 12	78 ± 9	81 ± 13	72 ± 4	46 ± 5	
P2	100 ± 10	91 ± 9	93 ± 10	99 ± 9	89 ± 6	97 ± 9	
P3	100 ± 7	82 ± 4	84 ± 8	72 ± 10	76 ± 8	89 ± 15	

P0 refers to the directly exposed cells and P1, 2 and 3 refer to the passage numbers at which the lethal mutation burden was measured in progeny without further exposure to radiation.

Table 4. Calculated contributions to the total dose of gamma and beta components of Ra-226 decay over the nine day exposure period for HaCaT cells (4a) or the 28 day period for CHSE-214 (4b).

(a) HaCaT

	^{226}Ra decay			Emission by daughters	
^{226}Ra Activity (Bq/ml)	α particles (mGy)	internal conversion (mGy)	X & γ photons (mGy)	^{214}Pb β^- decay (mGy)	^{214}Bi β^- decay (mGy)
0	0	0	0	0	0
0.1	0.06	9.18E − 06	4.94E − 07	0.0001	0.001
1	0.59	9.18E − 05	4.94E − 06	0.001	0.01
10	5.94	0.0009	4.94E − 05	0.01	0.06
100	59.37	0.009	0.0005	0.11	0.64
200	118.74	0.02	0.001	0.22	1.28
500	296.84	0.05	0.002	0.55	3.20

(b) CHSE-214

	^{226}Ra decay			Emission by daughters	
^{226}Ra Activity (Bq/ml)	α particles (mGy)	internal conversion (mGy)	X & γ photons (mGy)	^{214}Pb β^- decay (mGy)	^{214}Bi β^- decay (mGy)
0	0	0	0	0	0
0.1	0.18	2.85E − 05	1.54E − 06	0.0002	0.001
1	1.85	0.0003	1.54E − 05	0.002	0.01
10	18.47	0.003	0.0002	0.02	0.14
100	184.70	0.03	0.002	0.24	1.43
200	369.40	0.06	0.003	0.49	2.87
500	923.50	0.14	0.008	1.22	7.17

These results suggest that the approach of subtracting acute gamma exposure data from acute radium exposure data even in a range where biological effects are expected is too simplistic and does not yield meaningful information regarding the contribution of alpha radiation to the effect for either cell line.

The Monte Carlo model described in the methods section was then applied to the radium results. The results are shown in Table 4 and Figure 2(a,b). These data show the separate contributions in mGy of the alpha, beta and gamma components. It can be seen that the gamma component of dose is negligible and below that which could trigger lethal mutations or other NTE - 2mGy (Liu et al. 2006). The dose from beta decay of radon daughters is higher but only exceeds the 2mGy threshold for lethal mutation induction in the 500 Bq/ml exposure situation.

Discussion

At sub-lethal doses of gamma irradiation through acute exposure to ^{137}Cs, the HaCaT cell line displayed a region of low-dose hyper-radiosensitivity (HRS) followed by increased radioresistance (IRR). In addition, lethality was observed in subsequent generations (lethal mutation phenotype) with significant decreases in cloning efficiencies observed in uni-irradiated progeny cells. In contrast, only radioresistance was observed in the progenitor cells exposed to chronic ^{226}Ra with significantly higher survival and no observable region of HRS. Further, the observed progeny of these cells showed increased survival and lowered lethal mutation. In comparison, the results of experiments using the non-mammalian embryonic fish cell line showed the reverse of what was observed in human cell culture. Survival data following exposure to acute gamma irradiation confirmed existing radioresistance in the CHSE-214 cell line compared to human cell culture, with no significant lethality. However, survival data for cells exposed to ^{226}Ra suggested that alpha particles promoted lethality at doses otherwise known to have no significant effect. An aim of this paper was to determine to what extent the observed effects of ^{226}radium were due to radon decay chain elements contributing beta and gamma radiation to the biological material. Given the very different patterns of survival in directly irradiated cells and their progeny, it became apparent that comparison of

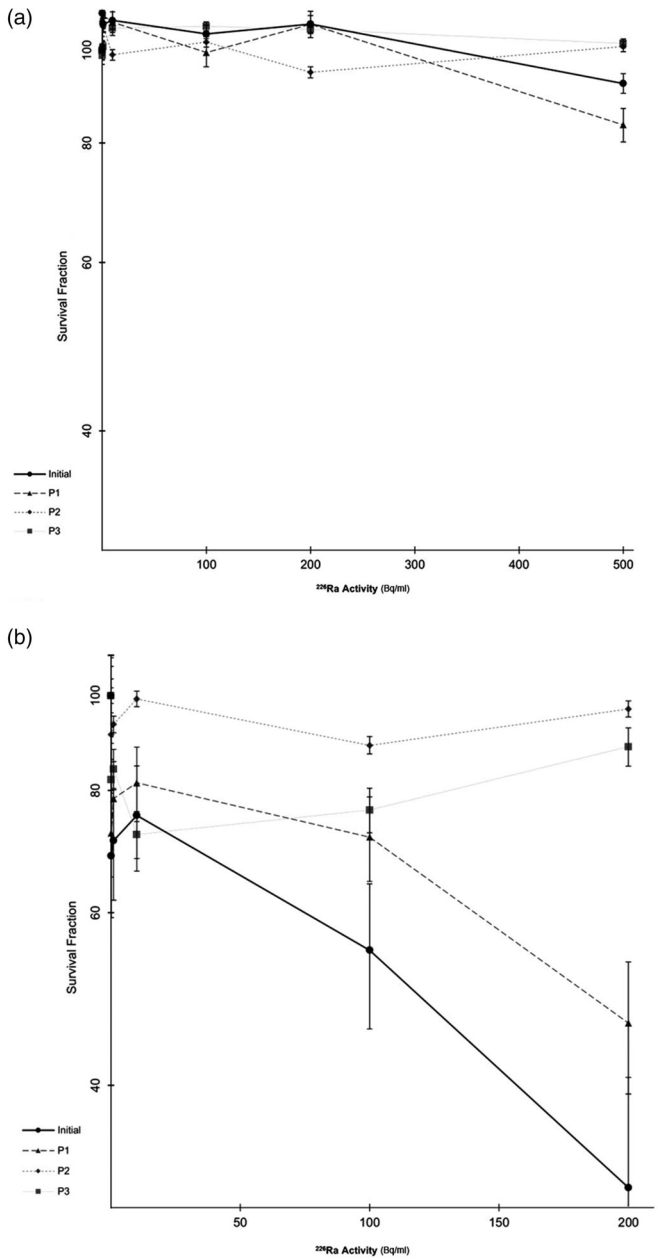

Figure 2. Surviving fraction (%) of initially irradiated and passage 1,2 and 3 progeny of HaCaT cells exposed to Ra-226 for 9 days (2a) and CHSE-214 cells exposed to Ra-226 for 28 days (2 b). The activity is shown in Bq/ml. The contributions of the decay products including radon daughters are shown on Table 4.

survival data for acute gamma exposure with mixed alpha/beta/gamma exposure would not yield useful data even if DDREF values were discounted. The Monte Carlo approach was used therefore to determine the individual components of dose in the culture flasks over the time of exposure to radium. Given the very low doses attributable to beta and gamma decay we conclude that the biological effects seen following ^{226}radium exposure in the flasks are most likely due to alpha radiation with a small contribution from beta radiation due to radon daughters. The results shows a marked difference in response of the human cell line (virtually no effect and a possible hormetic response), compared with the fish cell line (marked low dose hypersensitivity/induced radioresistance and persistent lethal mutation effects in progeny).

There are two important caveats when comparing these data:

1. There are significant differences in cell doubling times between CHSE cells and HaCaT cells. CHSE are a fish cell line and grow at 18 °C in a cooled incubator. They have a doubling time of approximately 127hrs (Plating efficiency 41.4 ± 2.2%) while HaCaT grow at 37 °C and have a doubling time of 23.6hrs (Plating efficiency 50.4 ± 0.8%). This difference will impact the average radiobiological effects (survival). It is probably best to regard the data as indicative of the likely complexities when extrapolating laboratory data to field situations or comparing likely effects of environmental exposures across cold and warm blooded species rather than as an absolute difference. Both the temperature difference and the doubling time difference would change DNA damage the repair kinetics (Mayer et al. 1987; MacLeod et al. 1990), The metabolic rate would also be different (Wheeler et al. 1992) although the Q10 of enzymes in poikilothermic versus homeothermic species show a remarkable ability of poikilothermic species to adapt to temperature swings while maintaining stable functions. (Robinson 2010). Either way the reality is that in the environment the ability of organisms to respond to radiation exposure will vary greatly depending on species and physical environment as well as dose.
2. In the experiments described above, an acute gamma exposure was used to try to determine the contribution of gamma alone to the total radium effect but this did not take into account dose rate effects, which are known to be important in low dose exposure scenarios. This means that the contribution of gamma should be taken as the maximum possible contribution rather than the actual contribution. An important point is that when the dose rate effect is examined for production of non-targeted effects (NTE) such as bystander effects or as examined here for lethal mutation induction, the literature suggests minimal impact of dose rate on NTE (Amundson et al. 2003; Belli and Tabocchini 2020). This probably relates to the fact that these are 'turned on' by very low doses (2-3mGy acute dose) (Liu et al. 2006) and the binary nature of the response means that once activated further exposure does not lead to increased effect (Schettino et al. 2005). So while the direct dose effect will be influenced by dose rate, the NTE may not.

Overall however it is important to note that temperature and growth rates for these cell lines do reflect the temperature optima of fish and mammalian species in nature.

Considering the potential for sub-lethal doses from chronic exposure to radium and its daughters found in waste products, to remnants of historic commercial and medical usage of radium (ranging from self-luminous paints to cancer treatment), the unconventional behavior observed

in both cell lines of this study have potential importance in radiological protection. Further, the presence of radium in waste reaching the ecosystem from mining and nuclear applications is important given the currently growing interest for non-human radiological protection.

The radioprotective quality of sub-lethal doses of alpha radiation that was observed in the HaCaT cell line, where cells displayed significantly lower lethality in the presence of alpha particles has not been noted previously in the literature. Current risk models for carcinogenesis and radiation protection at low doses do not consider effects like HRS/IRR and genomic instability phenomena, partly because the importance of such effects to the carcinogenic process have not been established. Research observing HRS/IRR behaviors suggest the activation of cell cycle checkpoints for increased cell repair, etc. as a possible mechanism for radioresistance (Fernet et al. 2010). Considering only radioresistance was observed in the presence of alpha radiation, the results suggest an ultra-low dose of alpha particles produces a sufficient level of genomic instability to activate the previously mentioned cell cycle checkpoints, inducing radioresistance. This effect was non-linear with dose with marked reduction as dose increased to the progenitor. The results observed in the CHSE-214 cell line on the other hand is in line with currently accepted descriptions on the effect of alpha particles at high doses. Here, the concentration of damage events is said to exceed a threshold at which effective repair becomes difficult (Blöcher 1988). Further lethality is seen in progeny as a de novo appearance of non-clonal lethal mutations, indicative of genomic instability. However, this decreases with subsequent generations suggesting the ability for existing damage repair mechanisms eventually to counteract the heritable susceptibility to lethal damage.

The results of the study support the need to consider dose-dependence when describing the effect of different radiation qualities. The results of the fish cell line experiments suggest a need to be aware of species differences and that protection for humans would not inherently protect ecosystems and non-human biota.

It should be noted that observed in vitro results cannot simply be translated to in vivo effects without much further research and discussion. For example, the evidence for heritable NTE through in vitro and non-human studies (Morgan 2012), is given appropriate context by epidemiological observations including germline mutation rates (Dubrova et al. 2002) and transgenerational genomic instability (Aghajanyan and Suskov 2009) observed in post-Chernobyl families. In addition, further research needs to be done to isolate the effect of dose rate during sub-lethal exposure to high-LET radiation, as differences in time for cell repair can affect the level of radioresistance observed.

Conclusion

At sub-lethal doses, survival greatly depends on repair mechanisms. Since the HaCaT cell line demonstrates hyper-radiosensitivity to acute gamma energy at low doses, high-LET alpha particle radiation may be able to produce sufficient genomic instability to induce radioresistance. In such instances, the ratio of biological damage caused by chronic alpha exposure is significantly lower compared with an equivalent dose of gamma energy alone, and as such a lower radiation weighting factor might be considered. However, while the CHSE-214 cell line demonstrates increased radioresistance to gamma energy, the concentrated nature of energy deposited causes increased lethality when exposed to alpha particles. These cases would suggest a higher radiation weighting factor, similar to what is currently recommended. Further study is required to isolate the effect of dose-rate at sub-lethal doses. In addition, further consideration is required to translate the observed in vitro results to in vivo effects.

In terms of the contributions of decay chain components of dose from the radium treatments, these appear to be negligible and below the threshold for inducing non-targeted effects except for the ^{214}Bi $\beta-$ decay for the highest applied ^{226}Ra activity.

Alpha-emitters are often found in environments and industrial applications related to the generation of nuclear energy. Further they can be dispersed during nuclear incidents and events of accidental release. As nuclear power grows as a potential carbon neutral solution to climate change, a shift from an over-simplistic model, to one that considers nuances at different exposure situations is crucial to provide meaningful risk management and radiation protection of human and non-human biota at low doses.

Disclosure statement

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References

Aghajanyan A, Suskov I. 2009. Transgenerational genomic instability in children of irradiated parents as a result of the Chernobyl Nuclear Accident. Mutat Res Mol Mech Mutagen. 671(1–2):52–57.

Amundson SA, Lee RA, Koch-Paiz CA, Bittner ML, Meltzer P, Trent JM, Fornace AJ. Jr. 2003. Differential responses of stress genes to low dose-rate irradiation. Mol Cancer Res. 1(6):445–452.

Belli M, Tabocchini MA. 2020. Ionizing radiation-induced epigenetic modifications and their relevance to radiation protection. IJMS. 21(17):5993.

Blöcher D. 1988. DNA double-strand break repair determines the RBE of alpha-particles. Int J Radiat Biol. 54(5):761–771.

Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol. 106(3): 761–771.

Browne E, Firestone RB, Shirly VS, editors. 1986. Radioactive isotopes. New York (NY): John Wiley & Sons.

Dubrova YE, Grant G, Chumak AA, Stezhka VA, Karakasian AN. 2002. Elevated minisatellite mutation rate in the post-chernobyl families from Ukraine. Am J Hum Genet. 71(4):801–809.

Eckert & Ziegler. 2010. Recommended nuclear decay data. Braunschweig: Eckert & Ziegler.

Fernet M, Mégnin-Chanet F, Hall J, Favaudon V. 2010. Control of the G2/M checkpoints after exposure to low doses of ionising radiation: implications for hyper-radiosensitivity. DNA Repair (Amst). 9(1): 48–57.

Fesenko S, Carvalho F, Martin P, Moore W, Yankovich T. 2014. Radium in the environment. In: The environmental behaviour of radium: revised edition technical reports series No 476. Vienna: IAEA; p. 33–105.

Goodhead DT. 1994. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int J Radiat Biol. 65(1):7–17.

Hoel DG. 2018. Nuclear epidemiologic studies and the estimation of DREF. Int J Radiat Biol. 94(4):307–314.

ICRP. 2007. 4.3 Dose quantities. In: The 2007 Recommendations of the international commission on radiological protection. Essen: Elsevier. p. 62–71.

Liu Z, Mothersill CE, McNeill FE, Lyng FM, Byun SH, Seymour CB, Prestwich WV. 2006. A dose threshold for a medium transfer bystander effect for a human skin cell line. Radiat Res. 166(1): 19–23.

Lyng FM, O'Reilly S, Cottell DC, Seymour CB, Mothersill C. 1996. Persistent expression of morphological abnormalities in the distant progeny of irradiated cells. Radiat Environ Biophys. 35(4):273–283.

MacLeod RA, Christie AF, Costa ND, Bryant PE. 1990. Repair kinetics in CHO cells of X-ray induced DNA damage and chromatid aberrations during a cell cycle extended by transient hypothermia. Mutagenesis. 5(3):279–283.

Marples B, Lambin P, Skov KA, Joiner MC. 1997. Low dose hyperradiosensitivity and increased radioresistance in mammalian cells. Int J Radiat Biol. 71(6):721–735.

Mayer PJ, Bradley MO, Nichols WW. 1987. The effect of mild hypothermia (34 degrees C) and mild hyperthermia (39 degrees C) on DNA damage, repair and aging of human diploid fibroblasts. Mech Ageing Dev. 39(3):203–222.

Miller RC, Randers-Pehrson G, Geard CR, Hall EJ, Brenner DJ. 1999. The oncogenic transforming potential of the passage of single alpha particles through mammalian cell nuclei. Proc Natl Acad Sci U S A. 96(1):19–22.

Morgan WF. 2012. Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro. Radiat Res. 178(2):AV223–AV236.

Mothersill C, Kadhim MA, O'Reilly S, Papworth D, Marsden SJ, Seymour CB, Wright EG. 2000. Dose- and time-response relationships for lethal mutations and chromosomal instability induced by ionizing radiation in an immortalized human keratinocyte cell line. Int J Radiat Biol. 76(6):799–806.

Nagasawa H, Little JB. 1999. Unexpected sensitivity to the induction of mutations by very low doses of alpha-particle radiation: evidence for a bystander effect. Radiat Res. 152(5):552.

O'Reilly S, Mothersill C, Seymour CB. 1994. Postirradiation expression of lethal mutations in an immortalized human keratinocyte cell line. Int J Radiat Biol. 66(1):77–83.

Puck TT, Marcus PI. 1955. A rapid method for viable cell titration and clone production with HELA cells in tissue culture: the use of X-irradiated cells to supply conditioning factors. Proc Natl Acad Sci U S A. 41(7):432–437.

Rühm W, Azizova TV, Bouffler SD, Little MP, Shore RE, Walsh L, Woloschak GE. 2016. Dose-rate effects in radiation biology and radiation protection. Ann ICRP. 45(1_suppl):262–279.

Robinson R. 2010. Firing hot, firing cold: how poikilotherms compensate for temperature swings. PLoS Biol. 8(8):e1000470.

Ryan LA, Seymour CB, O'Neill-Mehlenbacher A, Mothersill CE. 2008. Radiation-induced adaptive response in fish cell lines. J Environ Radioact. 99(4):739–747.

Schettino G, Folkard M, Michael BD, Prise KM. 2005. Low-dose binary behavior of bystander cell killing after microbeam irradiation of a single cell with focused c(k) X rays. Radiat Res. 163(3):332–336.

Seymour CB, Mothersill C, Alper T. 1986. High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation. Int J Radiat Biol Relat Stud Phys Chem Med. 50(1):167–179.

Thomas P, Tracy B, Ping T, Baweja A, Wickstrom M, Sidhu N, Hiebert L. 2007. Relative biological effectiveness (RBE) of alpha radiation in cultured porcine aortic endothelial cells. Int J Radiat Biol. 83(3):171–179.

Wheeler KT, Hickman R, Nelson GB, Moore SK, Wallen CA. 1992. Relationship between DNA damage, DNA repair, metabolic state and cell lethality. Radiat Environ Biophys. 31(2):101–115.