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

Chandula Fernando, Soo Hyun Byun, Xiaopei Shi, Colin B. Seymour, and Carmel E. Mothersill

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.

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.
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 radioreistance (IRR) in both cells surviving exposure to ionizing radiation and the progeny of these cells (Marple 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 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 assessed as reduced clonogenic 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 Heps 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% CO2.

The CHSE-214 is an embryonic cell line derived from Chinook salmon obtained as a gift from Dr. Neil 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 Heps 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 (Rica 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 226Ra were prepared using neutralized radium nitrate (Eckert & Ziegler 2010, Valencia, USA). 100 ml L-15 or RPMI medium was mixed with 1000 Bq of 226Ra solution. The concentration of 226Ra 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 $\gamma$-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 \( \gamma \) and electron absorbed doses from $^{226}$Ra and its daughters**

To compute the $\gamma$ 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 $10 \times 5 \text{ cm}^2$ 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 plastic layer. The cell layer was assumed to be a 0.1 mm thick sub-layer within 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.

From the list daughter nuclides, beta decay electrons from $^{214}$Pb 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 $10 \times 5 \times 2.5 \text{ cm}^3$, given a large fraction of $^{222}$Rn 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 $^{226}$Ra 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 $^{214}$Pb and $^{214}$Bi beta electrons, $^{214}$Bi beta dose is about six times higher than the $^{214}$Pb 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 $^{226}$Ra dose and the daughters’ beta dose, the daughters’ beta dose rate is about 70 times higher than the $^{226}$Ra 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
be done in the initial activity buildup phase for 214Pb and 214Bi.

**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 \( \alpha \)-particles on the survival and genomic instability of cells, this study examined the effects observed after \( \gamma \) irradiation (through acute exposure to 137Cs) and from mixed \( \alpha/\beta \) and \( \gamma \) 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 226Ra and 137Cs are shown in Table 2. Data for acute 137Cs 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% \((p = .004)\). 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 226Ra 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 226Ra 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 1.** Absorbed dose computed by MCNP for each type of the source card.

<table>
<thead>
<tr>
<th>Conversion electron</th>
<th>X and gamma</th>
<th>214Pb ( \beta^- ) decay electrons</th>
<th>214Bi ( \beta^- ) decay electrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.36 ( \times 10^{-14} ) Gy/decay</td>
<td>1.27 ( \times 10^{-15} ) Gy/decay</td>
<td>2.51 ( \times 10^{-15} ) Gy/decay</td>
<td>1.47 ( \times 10^{-15} ) Gy/decay</td>
</tr>
</tbody>
</table>

---

**Table 2.** Survival of HaCaT cells exposed to Cs-137 (2a) and Ra-226 (2b) 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 137Cs

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>0</th>
<th>0.05</th>
<th>0.1</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>100 ± 2</td>
<td>93 ± 6</td>
<td>90 ± 3</td>
<td>92 ± 6</td>
<td>86 ± 3</td>
<td>76 ± 2</td>
<td>68 ± 8</td>
</tr>
<tr>
<td>P1</td>
<td>100 ± 3</td>
<td>79 ± 7</td>
<td>75 ± 4</td>
<td>85 ± 7</td>
<td>83 ± 7</td>
<td>78 ± 4</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>P2</td>
<td>100 ± 3</td>
<td>77 ± 8</td>
<td>79 ± 3</td>
<td>90 ± 3</td>
<td>94 ± 6</td>
<td>96 ± 5</td>
<td>97 ± 9</td>
</tr>
</tbody>
</table>

(b) Survival fraction (%) HaCaT exposed to 226Ra

<table>
<thead>
<tr>
<th>Ra-226 (Bq/ml)</th>
<th>0</th>
<th>0.01</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>200</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>100 ± 1.5</td>
<td>109 ± 3.0</td>
<td>107 ± 3.8</td>
<td>107 ± 3.0</td>
<td>104 ± 2.7</td>
<td>107 ± 3.3</td>
<td>92 ± 1.4</td>
</tr>
<tr>
<td>P1</td>
<td>100 ± 2.6</td>
<td>108 ± 5.4</td>
<td>109 ± 8.2</td>
<td>107 ± 3.4</td>
<td>99 ± 6.0</td>
<td>106 ± 4.4</td>
<td>84 ± 3.0</td>
</tr>
<tr>
<td>P2</td>
<td>100 ± 2.1</td>
<td>101 ± 2.5</td>
<td>106 ± 3.3</td>
<td>99 ± 2.0</td>
<td>102 ± 2.7</td>
<td>95 ± 2.5</td>
<td>101 ± 2.4</td>
</tr>
<tr>
<td>P3</td>
<td>100 ± 2.8</td>
<td>99 ± 2.8</td>
<td>101 ± 3.6</td>
<td>105 ± 4.3</td>
<td>106 ± 3.5</td>
<td>105 ± 1.7</td>
<td>102 ± 2.7</td>
</tr>
</tbody>
</table>

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.
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 unirradiated 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

#### Table 3. Survival of CHSE-214 cells exposed to Cs-137 (3a) and Ra-226 (3b) 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.

<table>
<thead>
<tr>
<th>Activity (Bq/ml)</th>
<th>$x$ particles (mGy)</th>
<th>internal conversion (mGy)</th>
<th>$X$ &amp; $\gamma$ photons (mGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.06</td>
<td>4.94E - 07</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.1</td>
<td>0.59</td>
<td>4.94E - 06</td>
<td>0.01</td>
</tr>
<tr>
<td>1</td>
<td>5.94</td>
<td>4.94E - 05</td>
<td>0.06</td>
</tr>
<tr>
<td>10</td>
<td>59.37</td>
<td>0.009</td>
<td>0.64</td>
</tr>
<tr>
<td>100</td>
<td>118.74</td>
<td>0.02</td>
<td>1.28</td>
</tr>
<tr>
<td>500</td>
<td>296.84</td>
<td>0.05</td>
<td>3.20</td>
</tr>
</tbody>
</table>

#### 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).

<table>
<thead>
<tr>
<th>Activity (Bq/ml)</th>
<th>$x$ particles (mGy)</th>
<th>internal conversion (mGy)</th>
<th>$X$ &amp; $\gamma$ photons (mGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.18</td>
<td>2.85E - 05</td>
<td>1.54E - 06</td>
</tr>
<tr>
<td>1</td>
<td>1.85</td>
<td>0.0003</td>
<td>1.54E - 05</td>
</tr>
<tr>
<td>10</td>
<td>18.47</td>
<td>0.003</td>
<td>0.0002</td>
</tr>
<tr>
<td>100</td>
<td>184.70</td>
<td>0.03</td>
<td>0.002</td>
</tr>
<tr>
<td>200</td>
<td>369.40</td>
<td>0.06</td>
<td>0.003</td>
</tr>
<tr>
<td>500</td>
<td>923.50</td>
<td>0.14</td>
<td>0.008</td>
</tr>
</tbody>
</table>

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.
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 (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 and Suskov 2009) observed in post-Chernobyl families. In addition, further research needs to be done 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 $^{214}\text{Bi}$ $\beta$— decay for the highest applied $^{226}\text{Ra}$ 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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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.
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