Chromosome analysis in a case of a plutonium contaminated wound

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Abstract
Chromosome analysis of peripheral blood lymphocytes was undertaken over a 10 year period following an intake of plutonium through a hand wound. Frequencies of cells with unstable complex aberrations remained high throughout this time, probably reflecting direct exposure of lymphocytes as they passed plutonium which had transferred to regional lymph nodes. Analysis at the final sampling time also revealed cells with stable aberrations at a much higher frequency relative to the number of unstable cells than expected from direct exposure, and is therefore most likely to be reflecting exposure to lymphocyte precursor cells from plutonium that has become deposited on bone surfaces.

Keywords: plutonium contamination, chromosome aberrations, α-particle radiation

Westlakes Research Institute closed in 2010.

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1. Introduction

Plutonium is retained within the body, the pattern of deposition being dependant on the mode of entry. Following inhalation, it is primarily deposited in the liver and endosteal bone surfaces (Leggett et al. 2005). The regional lymph nodes are an additional important deposition site for plutonium which gains access to the body through a break in the skin (Schofield 1980). Once deposited it will remain for many years after the initial intake and will irradiate cells with densely ionising (i.e. high linear energy transfer) α-particle radiation (Zirkle et al. 1952, ICRP 2003). Chromosome analyses of peripheral blood lymphocytes from workers with known intakes of plutonium have shown that, where increases associated with α-particle exposure can be demonstrated, the predominant aberrant cell type is a stable cell with a simple translocation, these cells being descendants of bone marrow cells irradiated by plutonium deposited on the bone surfaces (Hande et al. 2003, Anderson et al. 2005, Sotnik et al. 2014, Curwen et al. 2015). Moreover, it has become apparent that when age and external γ-ray exposure are adequately considered, increases tend only to be observed when red bone marrow doses from α-particle radiation are of the order of 0.1 Gy or greater. In this respect, a recent reassessment of chromosome aberration data on plutonium workers from the Sellafield nuclear facility indicates that although increases in translocations have occurred, these can be accounted for by the associated external γ-ray exposure (Tawn et al. 2016). In contrast, in vitro studies have shown that chromosome aberrations induced by α-particle radiation are characterised by their complexity. However, the majority of aberrant cells containing complex aberrations will be unstable and will not be capable of undergoing cell division to give viable descendant cells (Anderson et al. 2003, Tawn et al. 2007, Curwen et al. 2012). Such cells arising in the red bone marrow from exposure to internally deposited plutonium will not survive to replenish the peripheral blood lymphocytes and therefore unstable complex cells observed in the mature lymphocytes tend to be rare and must have arisen by direct exposure. Over the decades of industrial uses of plutonium, there have been a number of cases of plutonium contaminated wounds and many of these have been hand wounds. Here we report a case of a worker with a plutonium contaminated hand wound who continued to exhibit high levels of peripheral blood lymphocytes with unstable complex aberrations for many years after the accident.

2. Materials and methods

2.1. Case history

The incident described here, a male worker who sustained a contaminated hand wound while working with plutonium, was first reported in the 1970s (Schofield and Dolphin 1974, Schofield et al. 1974). The amount of plutonium deposited was relatively large and the penetration deep, extending proximally and obliquely to a depth of about 1.5 cm down to the level of the deep palmer structures. Initial monitoring indicated the presence of approximately 14–15 μCi (518–555 kBq). Immediate exploration of the wound resulted in the removal of approximately 3.9 μCi (144 kBq) leaving roughly 10.3 μCi (381 kBq) at the wound site. During the following 15 days chelation therapy using diethylene-triamine-pentaacetic-acid (DTPA) and wound dressing changes resulted in the removal of another approximately 0.9 μCi (33 kBq). A subsequent wider excision of the wound on day 15 post-accident removed a further 6.9 μCi (255 kBq). It was estimated that approximately 1–2 μCi (37–74 kBq) remained in situ and following a skin graft the wound eventually healed. Cumulative external γ-ray exposure up to the time of the accident was 23 mGy and the
worker was then removed from any further radiation work. The worker died in his eighties, more than twenty years after the wound incident, from a non-malignant cause that is not thought, on the basis of current scientific knowledge, to be linked to plutonium exposure.

2.2. Chromosome studies

A peripheral blood sample was taken on six occasions over a 10 year period following the accident and cultured for 48 h using standard techniques for chromosome analysis (IAEA 2011). Cell culture and chromosome analysis on the first five samples was undertaken at the National Radiological Protection Board, UK. The fifth sample was also cultured and analysed at British Nuclear Fuels Ltd Sellafield nuclear facility, as was the final sample. Giemsa solid-stained slides were scored for unstable aberrations i.e. dicentrics, centric rings and acentrics. For five samples, 500 cells were scored, but for the penultimate sample 1000 cells were scored. Additionally, slides from the last two samples were G-banded with trypsin to a resolution of approximately 400 bands per cell (Seabright 1971) and 100 cells were karyotyped. In the G-banding analysis, cells with rearrangements involving one or two breaks were classified as simple whereas those involving three or more breaks in two or more chromosomes, resulting in multiple rearrangements, were recorded as complex. Cells with dicentrics, centric rings and acentric fragments were classed as unstable whereas cells with only monocentric chromosomes were classed as stable.

3. Results and discussion

Information on sampling times and cumulative internal red bone marrow doses is given in table 1. These doses are subject to considerable uncertainty since the urinalysis results on which they are based are known to have been heavily affected by chelation therapy using DTPA and no attempt has been made to compensate for the impact that this has on the dose assessment. Furthermore, the doses were calculated using the assumption that the wound represented direct injection of plutonium to blood and no attempt has been made to model the delayed transfer of material from the wound site to blood, that might be expected to occur in such circumstances, or the impact that this will have on doses.

Results of the Giemsa solid staining analysis are provided in table 2 and the distribution of the aberrations between cells in table 3. Table 4 presents the G-banding data.

Solid staining analysis only enables scoring of unstable aberrations and will give an indication of direct exposure to the peripheral blood lymphocytes. The background level of dicentrics is generally assumed to be 1 per 1000–2000 cells (Edwards et al 2007, IAEA 2011) and the solid staining findings at all sampling times greatly exceed this level. The distribution

| Sample number | Months after accident | RBM α-particle dose (mGy) |
|---------------|-----------------------|---------------------------|
| 1             | 12                    | 61.0                      |
| 2             | 45                    | 257.4                     |
| 3             | 54                    | 314.5                     |
| 4             | 59                    | 338.7                     |
| 5             | 84                    | 481.4                     |
| 6             | 124                   | 739.5                     |
of aberrations was tested for conformity with Poisson expectations. For a Poisson distribution a ratio of variance to mean of 1.0 is expected, with a value >1.0 indicating overdispersion. Thus, it is clear that the distribution of aberrations is overdispersed with the aberrations occurring in fewer cells than Poisson expectations dictate (table 3). Specifically, cells with two or more dicentrics accounted for approximately 46% of the total dicentrics observed. It also seems likely that cells with a single dicentric could contain stable rearrangements undetectable by solid staining since when a full karyotype is undertaken with G-banding all 12 of the dicentrics observed at 84 months and eight of the nine observed at 124 months were part of more complex rearrangements.

### Table 2. Aberration profile of solid stained samples at six sampling times following accident.

| Months after accident | Cells scored | Dicentrics (single dicentrics) | Acentric aberrations | Centric rings |
|-----------------------|-------------|--------------------------------|----------------------|--------------|
| 12                    | 500         | 14 (10)                        | 22                   | 1            |
| 45                    | 500         | 11 (6)                         | 11                   | 0            |
| 54                    | 500         | 11 (2)                         | 7                    | 0            |
| 59                    | 500         | 11 (11)                        | 12                   | 0            |
| 84                    | 1000        | 42 (16)                        | 42                   | 5            |
| 124                   | 500         | 25 (10)                        | 12                   | 0            |

### Table 3. Distribution of aberrations and total aberrant cell number in solid stained samples.

| Months after accident | Number of cells scored | Number of aberrations | Distribution of aberrations | Total aberrant cells | Ratio of variance to mean ± SE |
|-----------------------|------------------------|-----------------------|----------------------------|----------------------|-----------------------------|
|                       |                        | 1  2  3  4  5  6  7   |                            |                      |                              |
| 12                    | 500                    | 37                    | 10  7  3  1  0  0  0      | 21                   | 2.12 ± 0.062                |
| 45                    | 500                    | 22                    | 13  1  1  1  0  0  0      | 16                   | 1.87 ± 0.062                |
| 54                    | 500                    | 18                    | 4   3  1  0  1  0  0      | 9                    | 2.74 ± 0.062                |
| 59                    | 500                    | 23                    | 14  3  1  0  0  0  0      | 18                   | 1.48 ± 0.062                |
| 84                    | 1000                   | 89                    | 27  11 7  3  0  0  1      | 49                   | 2.51 ± 0.044                |
| 124                   | 500                    | 37                    | 10  6  2  1  1  0  0      | 20                   | 2.44 ± 0.062                |

### Table 4. G-banding aberration profile at 84 and 124 months following accident.

| Months after accident | Number of cells scored | Number of aberrant cells | Number of simple translocations (+ inversions) | Number of dicentrics (single dicentrics) | Number of stable simples | Number of stable complexes | Number of unstable simples | Number of unstable complexes |
|-----------------------|------------------------|--------------------------|-----------------------------------------------|------------------------------------------|----------------------------|---------------------------|----------------------------|----------------------------|
| 84                    | 100                    | 7                        | 3 (+1)                                        | 9 (1)                                    | 0                          | 0                         | 1                          | 5                          |
| 124                   | 100                    | 7                        | 3 (+1)                                        | 9 (1)                                    | 0                          | 0                         | 1                          | 5                          |

Note: N16
Three studies of plutonium workers using mFISH, which enables identification of all chromosomes and the identification of both stable and unstable aberrations, have demonstrated that the predominant aberrant cell type in peripheral blood lymphocytes is stable with a simple translocation (Hande et al. 2003, Anderson et al. 2005, Curwen et al. 2015). In the most recent study of four plutonium workers with cumulative red bone marrow α-particle radiation doses in the range 0.1–0.46 Gy, cells with a simple translocation comprised 73% of the aberrant cells (Curwen et al. 2015). Moreover, it could be demonstrated that the translocation yields were over and above those expected from the associated external γ-irradiation and the increases associated with the cumulative red bone marrow α-particle doses conform to expectations derived from in vitro dose response data.

G-banding analysis similarly allows identification of all chromosomes but, in contrast, the most common aberrant cell observed in the G-banding analysis of the wound case reported here is an unstable cell with complex aberrations. Indeed, it is the only aberrant cell type observed at 84 months accounting for all seven of the aberrant cells. A similar number of unstable cells were observed at 124 months but stable aberrant cells were also present. Of the 11 aberrant cells observed at 124 months, six were unstable and five stable. Five of the unstable cells were complex and one contained a single dicentric. Of the five stable cells, one was complex, three contained a single translocation and one contained an inversion.

Plutonium is retained long-term in the body and is also recycled through the blood from earlier deposition sites to other organs and tissues where, because of its long radioactive half-life, it continues to irradiate cells. Consequently, cumulative red bone marrow α-particle radiation dose from plutonium exposure increases over the 10 year period of this study. Expectations based on in vitro studies (Curwen et al. 2012) suggest that at 84 months the cumulative red bone marrow α-particle radiation dose of 482 mGy would result in ~2 translocations per 100 cells and by 124 months the dose of 740 mGy would result in ~3 translocations in 100 cells. Such exposure is not expected to result in unstable cells being present in peripheral blood lymphocytes since any such cells arising in haemopoietic precursor cells would not survive cell division. However, G-banding analysis indicates that unstable cells predominate. Nevertheless, the number of stable cells at 124 months relative to the number of unstable cells is much greater than expected from direct exposure (Curwen et al. 2012) and provides confirmation that the stable aberrant cells are most likely to be reflecting exposure of lymphocyte precursor cells from plutonium deposited in bone. The ongoing presence of high frequencies of unstable cells observed both with solid staining and G-banding suggests that direct exposure of the circulating mature blood lymphocytes to α-particle radiation has also continued in the 10 years since the accident. This exposure is unlikely to be from plutonium still retained in the wound and is more likely to occur as circulating cells encounter plutonium that has been distributed and retained elsewhere in the body. The movement of plutonium following a wound is predominantly to the regional lymph nodes (Schofield, 1980) and this is the most probable site of irradiation. To a lesser extent, circulating lymphocytes may also encounter plutonium deposited in the liver and bone.

The difficulty in interpreting chromosome aberration results in cases of incorporated radionuclides relates to uncertainties of the origins of the lymphocytes analysed and their likely site of irradiation (Ainsbury et al. 2014). In this case, where the mode of intake is well documented, the presence of unstable complex cells indicates continued direct irradiation of mature lymphocytes in the 10 years following the accident and the presence of stable aberrant cells in the final sample suggests additional irradiation of lymphocyte precursor cells. Plutonium deposited in the regional lymph nodes and the bone are considered to be the main sites of irradiation resulting in the chromosomally aberrant lymphocytes observed in this man.
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