Chromosome aberrations in workers occupationally exposed to tritium

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Abstract

This paper reports the findings of an historical chromosome analysis for unstable aberrations, undertaken on 34 nuclear workers with monitored exposure to tritium. The mean recorded β-particle dose from tritium was 9.33 mGy (range 0.25–79.71 mGy) and the mean occupational dose from external, mainly γ-ray, irradiation was 1.94 mGy (range 0.00–7.71 mGy). The dicentric frequency of 1.91 ± 0.53 × 10⁻³ per cell was significantly raised, in comparison with that of 0.61 ± 0.30 × 10⁻³ per cell for a group of 66 comparable worker controls unexposed to occupational radiation. The frequency of total aberrations was also significantly higher in the tritium workers. Comparisons with in vitro studies indicate that at these dose levels an increase in aberration frequency is not expected. However, the available historical tritium dose records were produced for the purposes of radiological protection and based on a methodology that has since been updated, so tritium doses are subject to considerable uncertainty. It is therefore recommended that, if possible, tritium doses are reassessed using information on historical recording practices in combination with current...
dosimetry methodology, and that further chromosome studies are undertaken using modern FISH techniques to establish stable aberration frequencies, as these will provide information on a cumulative biological effect.

Keywords: occupational exposure, tritium, chromosome aberrations

(Some figures may appear in colour only in the online journal)

1. Introduction

Tritium ($^3$H) is a radioactive isotope of hydrogen, with a physical half-life of 12.3 years. Tritium is a pure low energy $\beta$-particle emitter (with no accompanying $\gamma$-ray emission), and a radiation weighting factor ($w_R$) of one is recommended for electrons of all energies by the International Commission on Radiological Protection (ICRP) when calculating equivalent doses for the purposes of radiological protection (ICRP 2003). However, the low energy of the $\beta$-particle emitted by tritium (mean energy, 5.7 keV; maximum energy, 18.6 keV) produces a short track (in water the average length is 0.56 $\mu$m and the maximum length is 6 $\mu$m) that has a relatively high ionisation density per unit length when compared with $\gamma$-rays. This aspect of tritium, together with its incorporation in the organic molecules of biological tissues, has led to suggestions that the $w_R$ for tritium $\beta$-particles should be greater than one. For a discussion on this see the reports of the UK Advisory Group on Ionising Radiation (AGIR) (AGIR 2007) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (UNSCEAR 2016).

Tritium occurs naturally as a result of interactions of cosmic ray neutrons with nuclei in the upper atmosphere, but the consequent dose to humans is small, at 10 nGy per annum. Exposure to anthropogenic sources of tritium arises in a number of ways. Occupational exposure can occur in nuclear reactors using heavy water as a moderator and in tritium manufacturing and processing plants. A tritium processing plant at Capenhurst, Cheshire, UK, was operated between 1965 and 1987, initially by the UK Atomic Energy Authority (UKAEA) and from 1971 by British Nuclear Fuels Ltd (BNFL), but because the tritium handled was for use in nuclear weapons the existence of the facility was security classified during this operational period, and its existence at Capenhurst was publicly acknowledged only in 1997 (Jackson et al 1997). Blood samples had been taken from workers with a history of working at the Capenhurst tritium facility for an analysis of chromosome aberrations in lymphocytes, but the results of this study were not published in the open scientific literature because of the secrecy of the plant operations.

The quantification of chromosome aberrations in peripheral blood lymphocytes has proved to be a powerful technique for quantifying exposure to ionising radiation (International Atomic Energy Agency (IAEA) 2011). The presence of unstable chromosome aberrations, particularly dicentrics, is a well-established marker of recent exposure, and more recently developed techniques of fluorescence in situ hybridisation (FISH) chromosome painting enable the evaluation of stable translocations which can be used to indicate total cumulative dose. In light of the continuing debate on the biological consequences of tritium exposure and the interest in potential health effects (AGIR 2007, Melintescu et al 2007, Little and Lambert 2008, Little and Wakeford 2008, UNSCEAR 2016), we present the previously unpublished data on chromosome aberrations in lymphocytes from workers with recorded exposure to tritium at Capenhurst. This chromosome study was completed before the onset of FISH translocation analysis and was confined to the study of unstable aberrations.
2. Methods

The radiation worker group comprised 34 workers from the tritium facility at Capenhurst. The control group comprised 66 new entrants to the nuclear fuel reprocessing installation at Sellafield, Cumbria, UK, who had no known previous occupational exposure to radiation. At the time of the study, both establishments were operated by BNFL. The control group has been reported previously as part of other contemporaneous studies (Tawn 1987, Tawn and Binks 1989, Martin et al 1991). The two worker groups were studied for chromosome aberrations in the 1980s, using standard techniques available at the time. All participants gave informed consent at the time of blood sampling. Smoking status at time of blood sampling was recorded. External, mainly $\gamma$-ray, doses were measured by film badges and tritium doses determined by urine analysis according to the standard methodology of the time. However, because of the classified nature of plant operations, tritium doses were not reported separately and a single whole-body dose was recorded, which was the sum of external and tritium doses. The original tritium urinalysis records were kept separate from other dosimetry records at Capenhurst, and were held in different formats and in different locations over time (with their precise location becoming unclear with the passage of time), making them difficult to find and interpret. Consequently, although considerable progress has been made towards assembling a useable urinalysis database for these workers, it has not yet been possible to reassess their tritium doses using more recent dose methodology. For this reason, the doses used are based on those originally calculated for entry into the dose record at the time.

It is currently understood that the methodology used to produce these tritium doses was as follows. No organic binding of tritium was considered, it being assumed to be entirely homogeneously distributed throughout body-water. This meant that the tritium concentration in urine samples would be the same as for body-water and any doses received would be, in effect, whole-body doses. Hence, the assessment of total body content of tritium was a simple matter of multiplying the concentration in urine by the volume of total body-water, which was assumed to be 40 litres, based on the ICRP ‘reference man’ (ICRP 1974). Doses were calculated from the total body content of tritium, and this process included a radiation weighting factor, called the ‘Q factor’, of 1.7. The results were recorded in rem (roentgen equivalent in man). For this study the recorded tritium doses in rem have been converted into absorbed doses in grey, with the Q factor removed.

Peripheral blood lymphocytes were cultured for 48 h according to standard techniques (IAEA 2011). A total of 200 first division cells from each tritium worker and 100 from each control worker were scored for asymmetrical aberrations, i.e. dicentrics, centric rings, and excess acentric fragments.

Exact confidence intervals (CIs) and $P$-values for aberration rate ratios (RRs) were calculated using the online package OpenEpi (http://openepi.com/Menu/OE_Menu.htm).

3. Results

Details of the results for the study groups are presented in table 1. The dicentric frequency for the tritium workers was significantly greater than that for the control workers: RR = 3.15 (95% CI: 1.07, 11.2) ($P = 0.036$). The frequency of acentrics did not differ significantly between the two groups: RR = 1.53 (95% CI: 0.78, 3.05) ($P = 0.22$). However, the
frequency of total chromosome aberrations was significantly higher for the tritium workers in comparison with the controls: \(RR = 1.84\) (95% CI: 1.06, 3.27) \((P = 0.029)\).

4. Discussion

Elemental tritium (HT) is a gas. It exhibits very little absorption by the body, either through the lungs or the skin, so that exposure usually results in trivial doses. However, in the occupational environment HT becomes incorporated in chemical compounds as a substitute for stable hydrogen (i.e. protium), most notably in water (particularly moisture in the air), in the form of tritiated water (HTO). Any HTO vapour in the air is rapidly and completely absorbed within the lung during respiration and is also absorbed through the skin, so these are the most common routes of significant tritium intake in an occupational setting. (The prohibition of eating and drinking in working areas usually limits the possibility of significant intakes by ingestion.) For exposure to HTO the biokinetic model promulgated in ICRP Publication 56 (ICRP 1990), which is currently used for most dose assessments, makes the assumption that 100% is immediately absorbed and that HTO, like other body-water, has a turnover half-life of ten days. This assumption about the biokinetics of HTO is well established and has remained fairly consistent since the first ICRP tritium model was presented in Publication 2 (ICRP 1959). However, the amount of tritium removed from HTO by natural biosynthesis processes to become tissue-bound, i.e. as organically bound tritium (OBT), and the retention time for such OBT in body tissues, has been the subject of considerable debate. Earlier tritium biokinetic models, such as those in ICRP Publications 2 and 30 (ICRP 1979), which formed the methodological basis of the historical dose assessments used for workers, did not include a component of OBT. The ICRP Publication 56 model, which is currently recommended for occupational protection purposes, predicates that only 3% of tritium intake becomes OBT, with a retention half-life of 40 days. This small fixed percentage of OBT reflects the scenario where the pool of tritium available for biosynthesis is available for only a short time, as HTO is rapidly cleared with other body water, which is the case following a single acute exposure. Using this model of tritium biokinetics, doses from OBT following exposure to HTO are practically negligible, i.e. less than 10% of the total dose. However, for

| Table 1. Data on age, smoking, radiation exposure and aberration frequencies in tritium workers and control workers. |
|---------------------------------|-----------------|-----------------|
| No. of individuals              | 34              | 66              |
| Mean age, years (range)         | 43 (24–64)      | 40 (27–59)      |
| Percentage of smokers           | 44.1            | 33.3            |
| Mean external dose, mGy (range) | 1.94 (0.00–7.71)| 0               |
| Mean \(^3\)H dose, mGy (range)  | 9.33 (0.25–79.71)| 0               |
| Mean years worked with \(^3\)H (range) | 6.25 (1–22) | 0               |
| No. of cells                    | 6800            | 6600            |
| No. dicentrics (+ centric rings)| 13 (+1)         | 4 (+1)          |
| Dicentrics ± S.E. × 10\(^{-3}\) per cell | 1.91 ± 0.53 | 0.61 ± 0.30 |
| No. acentrics                   | 22              | 14              |
| Acentrics ± S.E. × 10\(^{-3}\) per cell | 3.24 ± 0.69 | 2.12 ± 0.57 |
| Total no. chromosome aberrations| 36              | 19              |
| Chromosome aberrations ± S.E. × 10\(^{-3}\) per cell | 5.29 ± 0.88 | 2.88 ± 0.66 |
more protracted exposure to HTO, animal studies have shown that up to 35% of tissue-bound hydrogen can be exchanged with tritium (NCRP 1979), and this could therefore make a significant contribution to dose. Moreover, when mice were given tritiated drinking water, OBT levels rose to 22% of body HTO after 56 days (Rodgers 1992). In contrast, human studies of HTO intakes have indicated similar patterns of uptake for acute and chronic exposure with, in both cases, the dose contribution from OBT being <10% of the body-water dose (Trivedi et al 2000). Information on HTO biokinetics that has become available since the ICRP Publication 56 model was promulgated has been incorporated into a new ICRP HTO model which has recently been published as ICRP Publication 134 (ICRP 2016). As with all recent ICRP biokinetic models, the Publication 134 model aims to reflect biological processes more accurately, and it incorporates the recycling of material between tissue compartments. Under this new ICRP HTO model the majority of tritium is still removed from the body, with an initial biological half-life of ten days, but there are now two longer retained components of OBT with biological turnover rates that approximate to half-lives of 40 days and one year, respectively. (It should be noted that, as the new model incorporates recycling, the effective net elimination half-lives are a little longer.)

A review by Straume and Carsten (1993) found that for a variety of genetic endpoints, including the induction of chromosome aberrations in human lymphocytes, relative biological effectiveness (RBE) values for β-particles emitted by HTO were generally in the range of 1 to 3, with the highest values found for comparisons with γ-rays. The authors noted that most of the studies reviewed were animal or in vitro studies involving higher doses and dose rates than those usually received by radiation workers, and that therefore, because of dose and dose-rate effectiveness factor (DDREF) considerations, the RBEs derived from these experiments could underestimate the effectiveness of tritium exposures more commonly received by humans. However, subsequent studies on chromosome aberrations in lymphocytes irradiated in vitro found similar RBE values of 2.7 for acute exposure given at 5 mGy min⁻¹ and 2.0 for chronic exposure at a dose rate of 0.2 mGy min⁻¹ (Tanaka et al 1994).

A report by the UK’s AGIR (AGIR 2007) emphasised that track structure considerations indicate that the ionisation density of tritium low energy β-particles is greater than that of x-rays, which is in turn greater than that of γ-rays, leading to the expectation that the RBE of tritium β-particles is likely to be greater than one. The AGIR appraised the available literature and found that tritium β-particle RBEs were generally in the range of 1 to 2 when x-rays were used as the comparison radiation, and 2 to 3 when γ-rays were the reference radiation. The group concluded that a high energy γ-ray source should be used as a reference for RBE studies and that a value of 2 should be used for tritium epidemiology studies and retrospective dose assessments. Moreover, the report suggested that the ICRP should consider adopting a value of 2 as the radiation weighting factor for radiological protection purposes. In support of this, a critical re-examination of a restricted number of the most optimal studies indicated a tritium β-particle RBE of 1.17 with respect to chronic X-irradiation and 2.19 with respect to chronic γ-irradiation (Little and Lambert 2008). More recently, a report by UNSCEAR (UNSCEAR 2016) reviewed around 50 different estimates of RBE values in animals and animal cells. The values ranged from 1.0 to 5.0 and 0.4 to 8.0 for comparisons with γ-rays and x-rays, respectively, and there was a tendency for RBE values to increase with decreasing doses. Chen (2013) compared the dose mean lineal energies of electrons emitted by HTO and OBT with those of 60Co γ-rays and concluded that the RBE could vary from 1.3 to 3.5 for HTO, and 2.3 to 5.6 for OBT. Moreover, as noted by Melintescu et al (2007), there are still no direct data on RBEs for the low doses associated with nuclear facilities.

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The importance of cytogenetic analysis of peripheral blood lymphocytes for the identification of radiation-induced aberrations as part of medical surveillance of radiation workers has been recognised for over 40 years (Lloyd et al 1973). Until the 1990s this tended to comprise the analysis of unstable aberrations, such as dicentrics, which could be easily identified using a conventional block staining technique. However, although it was recognised that increased frequencies of dicentrics were associated with occupational radiation exposure, it was difficult to quantify this effect. Buckton et al (1967) were among the first to recognise that this was because of the finite life of peripheral blood lymphocytes containing unstable aberrations. Subsequent studies suggested that cells with dicentrics are eliminated from the peripheral blood with a half-life of about three years (Edwards et al 2007). Haematopoietic precursor cells in the bone marrow containing dicentrics will not pass through repeated cell division and there will be no constant replenishment of the lymphocyte pool with descendants of irradiated cells containing dicentrics. Therefore, dicentrics, whilst a good indicator of recent exposure, are of limited use as a quantitative biomarker of chronic or historical exposure.

In addition to working with tritium, all tritium workers in this study were monitored for exposure to external sources of radiation. Considerable data are available suggesting that for high energy 60Co γ-irradiation the linear component of the dose response, which is dominant at low doses, is in the range 1–2 × 10⁻² per cell per Gy for dicentrics (Edwards et al 2005). The cumulative external doses for the tritium workers in this study ranged from 0 to 7.71 mGy, with a mean of 1.94 mGy, and are not therefore expected to influence dicentric frequencies. It is also unlikely that the slight difference in smoking profiles between the two groups is responsible for the difference in dicentric frequencies. Two large international studies on background translocation frequencies fail to agree on the influence of smoking, with one finding no effect (Whitehouse et al 2005) and the other reporting a RR of 1.19 for smokers versus non-smokers (Sigurdson et al 2008). Dicentrics and translocations are alternative methods of rejoicing following breaks in two chromosomes, but since translocations reflect cumulative genotoxic exposure and dicentrics reflect only recent exposure, it is reasonable to assume that the effect of smoking on dicentric frequencies is negligible. Moreover, no significant difference was found for the tritium workers between the dicentric frequency of 2.00 ± 0.82 × 10⁻³ for the 15 smokers and that of 1.84 ± 0.70 × 10⁻³ for the 19 non-smokers ($\chi^2 = 0.02, P = 0.88$).

Examination of the distribution of aberrations revealed that the 13 dicentrics in the cells of tritium workers occurred in 11 cells, with two cells each containing two dicentrics, one in a non-smoker with a tritium dose of 7.36 mGy and external γ-ray dose of 2.28 mGy, and one in a smoker with a tritium dose of 3.19 mGy and external γ-ray dose of zero. Such overdispersion can be indicative of exposure to high linear energy transfer (LET) radiation or high localized external γ-irradiation. However, the men in this tritium worker study were specifically chosen for not having been monitored for uranium and therefore not having worked in uranium enrichment plants. Owing to the nature of nuclear operations at Capenhurst, exposure to γ-rays from external sources was low (McGeoghegan and Binks 2000), hence the low recorded external doses (mean, 1.94 mGy) for this group of tritium workers.

Few studies have been reported on chromosome aberrations in workers exposed to tritium. Of most relevance to the present study of workers exposed chronically within permitted limits is that of Joksic and Spasojevic-Tisma (1998), who examined frequencies of dicentrics, rings and acentric fragments in two groups of tritium workers. The first group, who had been employed as dial painters of radioluminous timepieces and who had measurable concentrations of tritium in urine, had significant increases of dicentrics and total unstable aberrations. Chromosome aberration frequencies correlated more closely with the concentration of tritium in urine than

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with duration of occupational exposure, a not unexpected finding since dicentrics are a measure of recent exposure. The second group, who were employed in the weapons industry and had no detectable tritium in urine, demonstrated no significant increase in aberrations.

Although the increase in the frequency of dicentrics observed in the tritium workers in this study must reflect recent exposure, the mean cumulative β-ray dose from tritium for the group is only 9.33 mGy, and the mean dose for the three years prior to sampling is only 1.17 mGy. Reference to the in vitro dose-response curve for the induction of dicentrics in lymphocytes by HTO of $5.37 \pm 0.58 \times 10^{-2}$ per Gy reported by Prosser et al (1983) indicates that even an acute dose of 1.17 mGy would have a negligible effect. The increase in dicentrics and their cellular overdispersion, observed in the tritium workers in this study, is therefore unexpected. Furthermore, we are unaware of any additional genotoxic exposure which could account for this increase.

In light of the continuing interest surrounding the derivation of uptake and retention models for chronic tritium exposure and the appropriate radiation weighting factor for tritium β-particles, the following recommendations are made. Firstly, further efforts should be made to recover, if possible, the urinalysis monitoring data for Capenhurst tritium workers and to reassess the doses for those workers included in this study, using the best available methods and models. This would permit more reliable analyses to be conducted of potential dose-response relationships, and any enhanced effects due to radiation type. Secondly, further studies should be undertaken on tritium workers, using FISH techniques to establish stable aberration frequencies, since this will provide information on cumulative biological effect, which is considered potentially more relevant to risk. The UK AGIR (2007) recommended further studies of tritium workers, not only in the UK but internationally, and this study provides further incentive to conduct such studies.

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