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The Use of FISH Techniques for Retrospective Biological Dosimetry (COD)

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(summary)

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Introduction

An important outstanding problem in radiological protection is to assess the radiation risk at low doses and dose rates. There are very few direct human data to support an estimate. Present estimates rely upon an extrapolation of data from high doses and high dose rates using simple models of unproven validity. One way to obtain more direct evidence is to study epidemiologically populations that have been exposed above normal background levels over a long period of time. Radiation workers and members of the general population who live in areas of high activity are possible groups that could be studied.

One necessary parameter is dose to the individuals involved in such a study. At present techniques involve personal monitoring in the case of radiation workers or dose reconstruction for a population in a contaminated area. The reliability of these measurements is open to question. Personal monitors can be exposed without being worn and a person can be exposed without wearing a monitor. The memory of a person's movements over many decades could be suspect. A method of biological dosimetry, which acts as a permanent record of the dose to the individual, could be very useful.

The most sensitive biological dosimeter known to date uses the observation of dicentric chromosome aberrations in a particular sub-population of white cells in blood, which are called T-lymphocytes. They have a low yield in people not exposed above natural background levels and a dose of about 0.1 Gy of gamma rays can be detected. The system is used routinely to help investigate real or suspected radiation accidents often providing reassurance to a possible victim. The disadvantage of the dicentric assay is that it does not have a long-term "memory". The yield of dicentrics decreases with time after exposure. An aberration related to the dicentric, called the translocation, does not suffer this decrease. In the past, these translocations have been difficult to see but the recent ability to specifically highlight different chromosomes by "painting" them with fluorescent dyes has made translocation scoring much easier. Many cells may now be scored in a relatively short time. The technique is called fluorescence *in situ* hybridisation or FISH for short. This opens up the possibility to use translocation scoring in blood lymphocytes as a retrospective biological dosimeter. The question under investigation is how to use the technique in the most efficient way.

A previous contract studied which chromosomes to paint, which aberrations to score, their persistence in the human body with time, and their level in persons not irradiated beyond typical radiation background levels. All laboratories had chosen to paint three of the larger chromosomes and claimed efficiencies in the range 30-35 %. Despite some earlier indications to the contrary, it appeared that the choice of the chromosomes to paint was not critical. From studies of some persons exposed accidentally, either simple two-way translocations or the sum of one-way and two-way translocations were the aberrations of choice. At that stage it was undecided how to account for cells containing complex aberrations.

Progress in establishing how persistent these aberrations were was difficult because contradictory results were being obtained. Some experiments claimed good persistence while others showed a significant reduction. Further follow-up of a radiation accident to four persons in Estonia and to some ten people in Turkey was indicated. These are very important because they were the only two accidents of recent times that could be followed from the beginning by the FISH technique. The success of the previous contract was in establishing control levels of aberrations as a function of age for both two-way translocations and the sum

of the one- and two-way varieties. One worrying feature was the extra variation between persons even within fairly narrow age bands and this clearly required further investigation. So at the beginning of this Concerted Action project some outstanding questions still remained.

Objectives

The objective of this project was to provide answers to outstanding questions from the previous project and to eventually come to a consensus view on how to use FISH painting for retrospective biological dosimetry. Specifically control levels would be investigated further in an attempt to find out why some people had an unusually high or low yield of translocations. The survivors of the radiation accidents in Estonia and in Turkey would be followed further to give more information about persistence of translocations with time. There was a need to check that the different laboratories involved in this project were scoring consistently and so a small-scale intercomparison was organised. Any other experiments which contributed to the study of the persistence of translocations were also reported, noted and discussed. The final objective was to make an overall assessment of the utility of FISH techniques for retrospective dosimetry.

Results

The results can be divided into six parts:

a) Control levels

The very strong effect of age on average translocation yield in people not exposed to radiation other than background levels continued to be observed. Within age groups some laboratories found no persons with exceptionally high or low translocation yields. Some laboratories reported a few persons with exceptionally high yields but there was nothing to indicate why this was so. Those who live in very high radon areas appear to have a measurable increase in translocation yield but not in dicentrics. This is thought to reflect the higher exposure to the bone marrow than to other organs apart from the lung. There was one person that showed an exceptionally high translocation yield but further investigation showed this to be an unfortunate statistical observation.

b) The Estonian accident

There were four people who could be followed up after this accident and samples were shared between four laboratories. The accident has now been followed for 8 years. Three of the most heavily exposed cases have shown an initial decrease in translocation yield followed by a levelling off after about 2 years. The decrease in yield between the first sample and the final level was about 20-30 %. The largest decrease occurred for a person having the most non-uniform exposure. The fourth person had a lower exposure to radiation compared with the other three and showed no observable decrease in translocation yield over the 8 years. Following a suggestion that came from follow-up work for the Turkish accident (see later), translocation yields in guaranteed stable cells were measured. For the three most heavily exposed persons the translocation yield in stable cells only was independent of the time after the accident. In other words the initial decrease, reported above, disappeared. The prime cause of the change was the elimination

of some of the one-way translocations. This observation is very important for the final recommendations, see (f) below.

A further conclusion from this work was that we were able to effectively intercompare the scoring from four laboratories. Despite the employment of four different chromosome combinations, the resulting yields agreed well between laboratories.

c) The Turkish accident

In this accident there were five heavily exposed and five more lightly exposed persons. Three laboratories were involved in analysing samples obtained by a laboratory in Istanbul. Samples were taken up to 21 months following the accident, but after that we heard that the victims were no longer willing to give blood. Up to 21 months, translocation yields in the five most heavily exposed persons decreased by about 20 or 30 % with time. A chance observation was made by one laboratory, that if scoring was restricted to cells that did not contain a dicentric or an acentric or a centric ring of any kind, that is stable cells, the translocation yield was constant with time after the accident. This single, important observation caused an immediate attempt to check the principle with other cases, particularly the Estonian accident, see (b) above. The opportunity to intercompare scoring from the three laboratories involved in this work resulted in reasonable agreement.

d) The intercomparison

An attempt was made to organise an intercomparison between all seven partners in this project. It was decided to take advantage of an international criticality exercise to specifically test FISH scoring within the laboratories of this project. In the event, the two lowest dosed samples were chosen but one had too many cells containing complex aberrations to be useful. For the other, most laboratories reported a failure to produce preparations on microscope slides of sufficiently good quality for scoring using FISH. The results that were produced, however, showed good agreement between laboratories.

e) Persistence of translocations

Under this section many experiments which contribute to our knowledge of persistence of translocations are reported. During an experiment aimed at measuring translocations in residents of Belarus and Ukraine, one laboratory observed an increased translocation yield in two persons selected as controls. Further investigation showed that these two people were formerly given radiotherapy treatment but had chosen not to declare it when volunteering for the study. One was irradiated 5 years previously and the other 35 years previously.

Another laboratory re-examined a group of radiotherapy patients whose translocation yield had been measured for several years after treatment. They found a decline in yield with time, which they associated with partial body irradiation. However translocations in stable cells were constant with time. This lends further support for the conclusions reached under (b) and (c) above.

Another laboratory used irradiated monkeys to investigate persistence and obtained similar results to those reported under (b) and (c).

f) Final recommendations

The measurement of translocations in human lymphocytes is capable of providing an estimate of the lifetime dose to an individual. Strictly, it is the average bone marrow dose that is measured. The lower limit of sensitivity is dependent on the age of the sampled person and lies in the range 0.3 to 0.5 Gy of gamma rays. The lower limits for other radiation qualities are lower when measured in Gy. At present, calibration depends on comparison with measurements of dicentric yield because the accuracy of determining the relationship between translocation yield and dose at low doses and dose rates could be improved. The observation that translocation yield in stable cells is independent of the time of sampling implies that direct calibration should use stable cells only. On the few occasions that dose has been estimated by this technique, reasonable agreement has been achieved with the known or suspected circumstances of the irradiation.

Implications for radiation protection

A method has been developed to measure total lifetime dose to an individual from a simple blood sample. The method is still relatively expensive because it requires the scoring of about 3000 cells in an optical microscope. This would take a skilled scorer several days. The technique can be used in any situation where an exposure is suspected and occurred more than say 3 years previously. Total cumulative doses of more than about 0.3 Gy can be detected. This should be seen in the context that in a lifetime an average person will receive about 0.1 Gy from natural background radiation. Applications to estimate dose to groups of people require careful thought because there is a strong dependence of translocation yield with age in blood lymphocytes from unexposed persons. It is possible to use the technique as an aid to determine doses to individuals in epidemiological studies because the age of the individual will be known.