NON-TARGETED AND NON-LINEAR IONISING RADIATION EFFECTS IN BIOLOGY AND MEDICINE

Heriott Watt University, Edinburgh
8th – 10th August, 2006

This workshop was designed to bring together scientists and clinicians from different backgrounds who had an interest in biological effects of radiation. This included radiobiologists, radiochemists, epidemiologists, physicists, immunologists, biologists and radiotherapists to provide different perspectives on the topic and allow cross-fertilisation of ideas.

Genomic Instability and the Bystander Effect

The first presentation was given by Bill Morgan from the University of Maryland, who reviewed the radiation induced genomic instability in the bystander effect. Genomic instability is damage that may manifest in the progeny of cells that have been irradiated, so that subsequent cells generations maintain a memory of the irradiation. The cells affected continue to replicate but the genome starts to re-arrange during clone expansion. This may lead to a variety of effects such as delayed reproduction or cell death, and an increased level of reactive oxygen species, but there is no obvious mutator phenotide instability. Effects are observed at relatively high frequencies 3% per gray. Such chromosomes may be found in cell populations many years after irradiation. They may also occur not only in cells that have been irradiated directly but also in bystander cells.

Bystander effects occur in the neighbours of cells that have been irradiated and are communicated through cell-gap junctions. They can take many forms and include changes in gene expression, mutations, transformation, micro-nuclei and cell killing. It is as yet unclear what factors are involved in transmission of the signals between cells but it appears that not all cells can produce the factors and not all are receptive to them. Although the majority of experiments have been carried out with high LET alpha particle irradiations, bystander effects have been demonstrated with low LET radiations as well. The effects may be both bad in terms of genetic damage or cell killing, or good in terms of increased proliferation or radio-protective effects. Bystander effects have also been demonstrated in-vivo in the form of chromosome instability in the progeny of non-irradiated mice. Bystander effects occur at relatively low doses and the effects can be as significant at doses of 1 mGy as at 100 mGy. The effect is not dependent on irradiation of the nucleus and may be triggered by radiation of cell-cytoplasm. It is likely that there are multiple pathways involved in the signalling process.

The next talk of the session was given by Uri E Dubrova who described transgenerational radiation induced genetic instability. These experiments involved studies of mice whose parents had been irradiated in which there was genomic instability and an elevated mutation rate. Spontaneous damage in the DNA in the form of single and double strand breaks was observed in the offspring. Studies were also reported in which the offspring showed a higher incidence of cancer when exposed to carcinogenic agents than normal controlled mice.

Protective Mechanisms and Adaptive Responses

The final talk of the morning session was given by Ron Mitchell from AECL, Chalk River, USA, who described the adaptive response of protective low dose effects. He described how a stress response could be induced that protected against deterministic effects of radiation or another agent. This could be induced by radiation exposure but equally well by thermal stress or other agent. The initial experiments described were performed on cultures of yeast in which a reduced mutation frequency was observed in cultures irradiated with a high radiation dose that had been pre-exposed to a lower dose. The effect has been observed in single cells, insects, plants, lower vertebrates, human cultured cells and in mammals. The effect is observed at doses between 1 and 500 mGy and cells do not need to have been irradiated directly so that the response can occur through the bystander effect. Dr Mitchell reported results from a variety of experiments, including ones on frogs which have been

continually exposed in ponds by low-level tritium and mice in which the frequency of neoplastic transformation from a 4 Gy exposure have been decreased by delivery of a 100 mGy dose 24 hours beforehand. In other experiments, similar protective effects could be induced by a thermal stress putting the mouse at a temperature of 40.5^{o}C for an hour before the radiation exposure. Experiments were also performed on cancer-prone mice which developed lymphomas and in these it was found that a 10 mGy exposure delayed the onset of tumour development but with a higher dose of 100 mGy there was no delay. Similar results were demonstrated in mice with osteosarcoma, while experiments on rats to whom the carcinogen MNNG was administered showed a decrease in skin cancer incidence by a factor of 5 following an exposure of 0.5 Gy of beta radiation. Another effect that he reported was a delay in the onset of acute ulcerated dermatitis in older mice given chronic exposures of 0.3 mGy per day.

Polly Matzinger from NIH, Bethesda, USA, started the afternoon session with a talk entitled "The danger model: communication between tissues of the immune system". She provided a layman's description of how the immune system works using B-cell which react by picking up pieces of bacteria or viruses and developing surface reactors to neutralise these bacteria or viruses. These can replicate rapidly to protect the cell. However, this is not the whole story, there are T-helper cells which help to initiate the process and a third presenter-cell involved in the initial stimulation of the immune process. Initiation of the response requires a signal from a cell that is damaged or dying. A cell that dies through apoptosis will not provide the signal. Dr Matzinger described how the response was triggered by damaged cells produced in transplantation but that that would not necessarily be triggered by a tumour as in the early stages the cells are usually healthy and not necrotic. Tumour cell growth is actually similar to the healing process. The immune response is triggered for a limited period and so she indicated that injections developed to fight cancer would require to be injected repeatedly in order to continue triggering the immune response. For the essence of the immune response is that it requires damaged or dying cells to send the signal which provides the initial trigger. Radiation which kills the cells through apoptopic death will not trigger the immune response.

The next talk entitled "A sense of danger from ionising radiation" was given by Bill McBride from UCLA, USA. The talk revolved around the issue of whether radiation generates "danger" signals which are involved in pathogenesis in normal tissue damage. The main tissue responses to the radiation are DNA repair, cell cycle arrest, cell death through apoptosis, and tissue repair and regeneration. Results from many different studies were presented and discussed. Radiation induces a pro-inflammatory tissue response which is dependent on dose, time and strain of animals studied. This involves cytokines, infiltrating immune cells and changes in cellular phenotypes. Tissue repair of radiation damage affects the immunological landscape in many more ways than cell killing.

A talk on apoptosis was given by Seamus Martin from Trinity College, Dublin. Apoptosis is a mechanism used to remove cells that are no longer required. It occurs during homeostasis to deal with damaged cells and is important in maintaining cell numbers at the required level. Apoptosis involves packaging of the cells preventing spillage of the contents and so represents a more controlled cellular demolition than necrosis in which uncontrolled release of cell contents occurs. Apoptosis is co-ordinated by Cystein Aportic Acid specific proteases (CASPASES) and there are different pathways by which the response is triggered. The time before this response is triggered may be variable, but once it is activated, cell death takes a fairly constant time.

<u>Cancer and other Effects on Humans</u>

The next talk, by Fran Balkwill from the Barts and London Queen Mary's School of Medicine, looked further at links between inflammation and cancer. Many cancers arise at sites of chronic inflammation and effective cells of chronic inflammation are found in cancers. The influence of various chemical factors was discussed. TNF-alpha is a key cytokine in inflammation when produced by cells with malignant potential. It may promote and amplify the progress of a tumour. Studies have shown that reduction in TNF-alpha does not stop cells developing but does stop their spread and migration.

A discussion session was led by Professor Eric Wright from Dundee University about the implications of topic discussed during the day. Mark Little from Imperial College, London, reviewed the evidence for non-cancer pathologies induced by radiation. Cardiovascular and cerebral vascular disease have been observed in the A-bomb survivors, radiotherapy patients and Chernobyl recovery workers. Most information is available from the A-bomb survivors who have been assessed for these diseases since 1968. There is an excess relative risk of between 0.12 and 0.18 per Sievert, but there was no evidence of any effect below 300 mSv. However, the errors in these assessments are large and it is impossible to draw any conclusion about the shape of the dose response curve.

Non-ionising Radiations and Chemicals

The next session in the conference considered effects of non-ionising radiation and chemicals. Carmel Mothersill from McMaster University, Canada, reviewed evidence relating to triggering of a bystander effect from chemical exposure. This is important at the present time because of concern about possible effects from low exposures from multiple pollutants. However, there are issues about how to test for a chemical and how any delayed bystander effect can be separated from direct exposure to the chemical. Examples of various studies were given. Hydroquinone has been shown to induce chromosomal instability, micro-nuclei and apoptosis and a similar level of effect appears to occur over a range of concentrations in a similar manner to the radiation related bystander effect. Studies have been carried out on cell lines using organic pesticides, detergents, chromium, which is used in hip implants and Vanadium. Production of serotonin was suggested as one possible mechanism for the effects observed. Carmel also described experiments on the radiation bystander effect involving a fish exposed to radiation and others placed in the same water in which subtle changes in certain tissues were observed.

The next talk was from Trevor MacMillan from Lancaster University, who described experiments in which the bystander effect was observed to occur after exposure of cell lines to UVA radiation with wavelengths between 340 and 400 nm. Cell plates were placed in the same culture media as cells exposed to UVA and the efficiency of formation of clonogenic groups of HaCaT cells assessed. A fall in the numbers was demonstrated following UVA irradiation but not UVB radiation. Effects from a similar UVA dose were found to be inversely related to the dose-rate of the exposure.

Dennis Henshaw from the University of Bristol described studies of electromagnetic fields from electrical supply and cancer induction. Recent reviews which have pulled data from many studies of childhood leukaemia suggest a possible link between 50/60 Hz magnetic field exposure and childhood leukaemia, with an exposure level of 0.3/0.4 μTesla, doubling the frequency of occurrence. The body of evidence indicative of a causal link is growing. Studies of adult leukaemia, brain cancer and motor neurone disease, as well as miscarriages suggest there is also a link for these diseases. Two hypotheses were put forward as possible explanations. One common factor in all the conditions that seem to be affected by magnetic fields is disruption of melatonin production. This is a hormone produced by the pineal land. Melatonin is produced mainly at night and the production is suppressed by exposure to light. Studies have been carried out suggesting a link between reduced melatonin levels and breast cancer. Magnetic fields down to 0.2 μTesla have been shown to depress melatonin production, so this could be a factor in increased leukaemia incidence. A second mechanism which might also be involved relates to pairs of free radicals, $H^{-} + OH^{-}$. These free radicals set up an equilibrium transition between a singlet and triplet state. The magnetic field changes the balance of the equilibrium and so the availability of free radicals to cause damage. The evidence suggests that the magnetic fields are involved in a promotional rather than an initiation mechanism and enhance the effect of other environmental carcinogens rather than initiating cancer development themselves. The free radical pair is an important mechanism involved in the sensing and magnetic fields by birds such as the robin and the pigeon. Radical pairs created by visible light which are present in the eye provide the bird with an internal compass.

In addition to the effects of magnetic fields, there may also be an effect from the electric field associated with the power lines. Ions are produced by a power line and current flows of the order of 0.1 mA per metre may be generated equating to 6.25×10^{14} charges per metre per second. The charge

cloud or corona can extend for 5-7 kilometres from the power lines themselves. Thus there may be components of both the magnetic and electric field properties which could potentially influence leukaemia development. The direct effect of the magnetic field itself would only extend for 50-60 metres from the power lines, whereas the corona of particles might typically start to increase about 100 metres downwind as charge diffuses down to ground level and might typically extend to about 600 metres. Thus the effect from the corona may extend much further than from direct exposure. No link can be drawn between exposure to these fields and those from MRI scanners, since the exposure described in these studies is at a different frequency and extends over a much longer period. Effects have also been reported in animal experiments but are dependent on strain and these appear to confirm that the electromagnetic fields play a promotional role.

Adaptation Selection and Evolution

A talk was given by Werner Mueller from University of Mienz, Germany, who described work on various sponges which were the first organism to embrace sexual reproduction and are in the direct revolutionary tree from which mammals developed.

Susan Rosenberg from Baylor College of Medicine in Houston, Texas, discussed DNA repair, genetic instability and adaptive mutation. She postulated that environmental stresses might induce mutations which might prove favourable for a changed environment. The mechanism might be that there would normally be a high fidelity of double-strand break DNA repair which under stress changes to an error prone double-strand break repair process which is more likely to lead to muto-genesis. Experiments were described in which cells were stressed through starvation to provoke growth limitation performed on cultures of E-coli cells. The genetic effect occurs in unselected genes and could provide possible mechanisms for group as well as individual evolution.

Bernie Strous from the University of Chicago gave a talk on Hyper Mutability and Silent Mutations in Human Carcinogenesis. He reported analyses of results on the 53 mutations database which now contains 21,000 reports of different mutations from the scientific literature. The mutations found tend to be clustered and have a high proportion of silent mutations.

Radiotherapy

The final few talks of the meeting looked at implications for radiotherapy. Kevin Prise from the Gray Cancer Institute gave a talk about the possible implications for bystander mediated cell deaths. Looked at what factors influenced the bystander effect and reported results from micro-beam studies. He concluded that the release of the bystander signal is independent of the irradiated cell phenotype and that it is the phenotype of the bystander cells that governs the response. Effects extended for a millimetre from the limit of the radiation beam, but distances could be greater and this could have important implications for radiotherapy treatments. Alistair Munro, an Oncologist from the University of Dundee, then gave a talk setting out the position of the radiotherapy clinician, the decisions they have to make and the bases for these. Following this, Marie Boyd from the Beatson Laboratories, University of Glasgow, gave a talk on the physical and biological bystander effects in targeted radiotherapy. She looked at bystander effects from iodine-123 which emits Auger electrons with a track width of 10 nm and reported studies on steroids. The final talk of the day was given by Alan Nahum from the Clatterbridge Centre for Oncology, The Wirral, concerning the implications of the bystander effect for radiotherapy treatment planning. He reported effects of exposure of cells in culture medium in a wedged field with a dose gradient. Experiments were carried out looking at cells from three separate compartments and differences were found depending on whether the cell culture medium was free to move between all three cells or whether they were completely separate. If bystander effect communication were to occur in-vivo in this way, it could affect tissues such as giving better sparing of normal tissue in the beam path and whether target dose uniformity was as important as it is normally considered in radiotherapy treatment planning.

Conclusion

The meeting concluded with discussions about the implications for the future of the things discussed. There was a strong feeling among a number of participants at the workshop that there was strong evidence that there was a threshold below which effects such as radiation carcinogenesis did not occur. However, the majority of the group acknowledged that the linear no threshold approach was the simplest to justify. If any change in the approach of ICRP was to be made in the future, this would require more evidence from animal models, since human epidemiological data does not have the statistical power to verify any safe dose threshold.