Plenary panel 1: The scientific bases of radiation protection

Non-targeted effects of ionising radiation - Implications for radiation protection

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Abstract

The universality of the target theory of radiation-induced effects is challenged by observations on non-targeted effects such as bystander effects, genomic instability and adaptive response. Essential features of non-targeted effects are that they do not require direct nuclear exposure by radiation and they are particularly significant at low doses. This new evidence suggests a need for a new paradigm in radiation biology. The new paradigm should cover both the classical (targeted) and the non-targeted effects. New aspects include the role of cellular communication and tissue-level responses. A better understanding of non-targeted effects may have important consequences for health risk assessment and, consequently, on radiation protection. Non-targeted effects may contribute to the estimation of cancer risk from occupational, medical and environmental exposures. In particular, they may have implications for the applicability of the Linear-No-Threshold (LNT) model in extrapolating radiation risk data into the low-dose region. This also means that the adequacy of the concept of dose to estimate risk is challenged by these findings. Moreover, these effects may provide new mechanistic explanations for the development of non-cancer diseases. Further research is required to determine if these effects, typically measured in cell cultures, are applicable in tissue level, whole animals, and ultimately in humans.

1. Introduction

A basic paradigm in radiobiology is that, after exposure to ionising radiation, the deposition of energy in the cell nucleus and the resulting damage to DNA, the primary target, are responsible for the harmful biological effects of radiation (Lea, 1946). The radiation-induced changes are thought to be fixed already in the first cell division following the radiation exposure and health effects are considered to result as a consequence of clonal proliferation of cells carrying mutations in specific genes (Ward, 1999; Prise, et al., 2005).

There is now accumulating evidence that challenges the universality of the target theory of radiation induced effects. These effects have been termed "non-(DNA)-targeted" (Morgan, 2003a) and include radiation-induced bystander effects, genomic instability, adaptive response, clastogenic factors, delayed reproductive death, premature differentiation of cells, low dose hypersensitivity and induction of genes by radiation. Essential features of non-targeted effects are that they do not require direct nuclear exposure by radiation and they are particularly significant at low doses. This new evidence suggests a need for a new paradigm in radiation biology (Baverstock and Belyakov, 2005). The new paradigm should cover both the classical (targeted) and the non-targeted effects. New aspects include the role of cellular communication and tissue-level responses.

A better understanding of non-targeted effects may have important consequences on the health risk assessment and, consequently, on radiation protection. The current paper gives an overview on the non-targeted effects, in particular bystander response and genomic instability. Furthermore, the potential implications of non-targeted effects on risk assessment and radiation protection will be discussed.

1.1 Bystander effect

Bystander effects are changes in cells that were not directly hit by radiation but were nearby (Nagasawa and Little, 1992; Mothersill and Seymour, 1997; Prise, et al., 1998; Belyakov, et al., 2001; Belyakov, et al., 2003; Belyakov, et al., 2005b). The signal can be transferred via the culture medium, "clastogenic factors" (Auclair, et al., 1990; Emerit, 1994; Emerit, et al., 1994; Emerit, et al., 1997), or cell-to-cell communication as inhibition of cell communication prevents bystander effects (Azzam, et al., 1998; Shao, et al., 2005; Yang, et al., 2005). Bystander effects have been described in a variety of cellular systems and in tissue explants.

Bystander effects are not new. Starting from the 1960's, there is extensive literature on clastogenic factors and other "compounds" that stimulate or modify responses in cells that were not damaged (Littlefield and Hoffmann, 1993; Emerit, 1994). Modern microbeam exposure systems capable of exposing single cells or even defined cellular organelles to charged particles or X-rays have facilitated research on bystander effects(Zhou, et al., 2000; Belyakov, et al., 2003; Shao, et al., 2003b; Zhou, et al., 2003; Ponnaiya, et al., 2004; Schettino, et al., 2005); see also reviews (Prise, et al., 2002; Hall and Hei, 2003; Osterreicher, et al., 2003). Such irradiation facilities also make it possible to target subcellular structures, such as nucleus, cytoplasm or mitochondria with either a single or an exact number of charged particles or exact doses of X-rays. The dose-effect relationship for bystander effect invariably shows a plateau below one Gray. Moreover, the effect appears to be determined by dose per hit cell, rather than number of cells hit, and high and low LET radiations appear to be equally effective. Bystander effects are the most likely drivers for the more delayed non-targeted effects such as genomic instability and adaptive response.

A variety of effects has been described in the bystander cells: increases or decreases in damage-inducible and stress-related proteins (Hickman, et al., 1994; Azzam, et al., 2001), increases or decreases in reactive oxygen (Iyer, et al., 2000; Morgan, et al., 2002; Shao, et al., 2005) or nitrogen species (Matsumoto, et al., 2000; Shao, et al., 2003b), cell death (Mothersill and Seymour, 1997; Schettino, et al., 2005) or cell proliferation (Iyer, et al., 2000; Shao, et al., 2003a), cell differentiation (Belyakov, et al., 2002; Belyakov, et al., 2005a), radio-adaptation (Kadhim, et al., 2004; Mothersill and Seymour, 2005), induction of mutations (Nagasawa and Little, 1999; Zhou, et al., 2000) and chromosome damage (Prise, et al., 1998; Belyakov, et al., 2001), genomic instability (Watson, et al., 2000; Watson, et al., 2001; Lorimore, et al., 2003; Lorimore and Wright, 2003; Moore, et al., 2005) and neoplastic transformation (Lewis, et al., 2001; Sawant, et al., 2001). Irradiation of cytoplasm has been shown to lead to a mutation in nucleus or in the bystander cell (Wu, et al., 1999) and the mutation spectrum in bystander cells shows point mutations instead of deletions (Zhou, et al., 2000).

Bystander effect has been shown to be induced by very low doses (Michael, et al., 2000), and it is induced by high-LET (Michael, et al., 2000; Belyakov, et al., 2001) and low-LET radiation (Prise, et al., 2003a). The dose response for bystander effect is non-linear, showing first a sharp increase and then a plateau at higher doses (Michael, et al., 2000; Belyakov, et al., 2001; Morgan, 2003b; Morgan, 2003a), see Fig. 1. Bystander effect is currently considered to be the most likely driver for genomic instability (Watson, et al., 2000; Watson, et al., 2001; Lorimore, et al., 2003; Lorimore and Wright, 2003; Moore, et al., 2005). Bystander effect has been recently demonstrated also in vivo (Watson, et al., 2001; Xue, et al., 2002; Lorimore, et al., 2005).

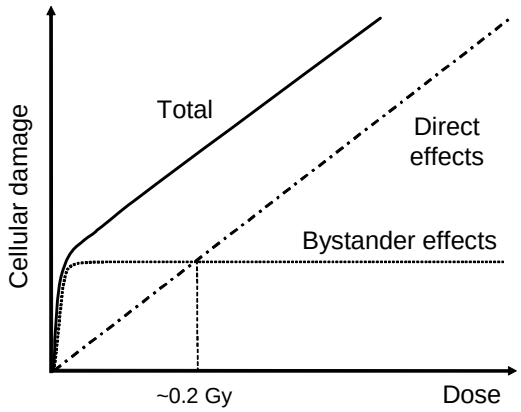

Figure 1. Contribution of bystander and direct component to the radiation induced damage (0.2 Gy is an estimation, based on published data from several groups).

1.2 Genomic instability

Radiation-induced genomic instability means that the progeny of irradiated cells show occurrence of new mutations and/or new chromosomal aberrations or other genomic damage for many generations (Kadhim, et al., 1995). Affected progeny also demonstrate high levels of lethal mutation, which may be measured as delayed reproductive cell death and/or delayed apoptosis. As lethal mutations cannot pass to the next cell generations, it is evident that they are induced de novo in cells that were not exposed to radiation (Kadhim, et al., 1994). Genomic instability occurs in the progeny of irradiated cells at a frequency that is several orders of magnitude higher than would be expected for a mutation of a specific gene (Suzuki, et al., 2003). Therefore a mutation in, for example, a repair gene is not a likely explanation for the induction of genomic instability. Genomic instability can occur both in the progeny of hit cells and bystander cells (demonstrated both in vitro and in vivo), see (Kadhim, et al., 1995; Watson, et al., 2000; Watson, et al., 2001; Lorimore and Wright, 2003; Lorimore, et al., 2005).

Genomic instability is induced both by high-LET and low-LET radiation (Limoli, et al., 2000; Hall and Hei, 2003; Smith, et al., 2003), but not all cell lines show this effect. No individual gene consistently related to induction of genomic instability in gene expression studies (Suzuki, et al., 2003). Genomic instability is induced by very low doses of ionising radiation, down to a single alpha particle (Kadhim, et al., 2001). The dose-effect relationship for genomic instability invariably shows a plateau but is a function of time at which effects are scored and there is no obvious dose-rate effect (Smith, et al., 2003; Preston, 2005). High LET is more effective than low LET, but LET also influences the temporal pattern of expression. The perpetuation seems to involve epigenetic mechanisms (Wright, 1998).

Individual sensitivity (genetic predisposition) plays a role both in genomic instability and bystander effect (Mothersill, et al., 2001; Belyakov, et al., 2003). Genotypes that have a more effective apoptotic response seem to be less predisposed to the development of malignancy and genotypes that have a less effective apoptotic response seem to be more predisposed to the development of genomic instability and malignancy (Watson, et al., 2001). Bystander effect and genomic instability are induced by high-LET and low-LET radiation, but not all cell lines show these effects (Mothersill, et al., 2002). Interindividual variation in bystander response has also been shown in human tissue (Mothersill, et al., 2001; Belyakov, et al., 2003; Mothersill, et al., 2005).

2. Implications for risk assessment and radiation protection

The system of radiation protection has a number of basic assumptions that are challenged by the non-targeted effects. The basic assumptions include the following: knowledge of radiation risk is based on direct epidemiological evidence, as well as scientific study of radiation biology; the system is designed to protect against both deterministic and stochastic effects; a linear, non-threshold (LNT) dose-response relationship is used for all long-term health effects (e.g. cancer, genetic effects); a dose and dose-rate correction factor is used to relate the effects of acute exposures to chronic exposures (DDREF); radiation dose is used as a surrogate for risk; the effects produced by different types of radiation are qualitatively the same doses can be summed to predict overall risk; the objective of the system is to protect the individual; the protection system is generally applicable, in the same fashion, to all age groups and to both sexes; and, the protection includes the principles of justification, optimisation and exposure restrictions. There is broad international agreement among governments that the current system of radiation protection is effective, robust and adequately protects man and the environment.

2.1. A shift in radiobiological paradigm

The current paradigm in radiobiology is that, after exposure to ionising radiation, the deposition of energy in the cell nucleus and the resulting damage to DNA, the primary target, are responsible for the harmful biological effects of radiation. Radiation-induced changes are thought to be fixed by the first cell division following the radiation exposure and health effects are considered to result as a consequence of clonal proliferation of cells carrying mutations in specific genes, or deleted and/or transposed sections of chromosomes. Since the initial damage induced in DNA has been shown to be a linear or linear-quadratic function of dose, risk is also be considered to be a similar function of dose, and frequently (for conservatism) risk is assumed to be a linear function of dose. In this case, risk from multiple exposures is considered to be additive, and risk from high and low LET radiation exposure is assumed to be qualitatively the same. These assumptions are incorporated into the Linear-No-Threshold (LNT) Hypothesis that is used in radiation protection practice.

There is now accumulating evidence that challenges the universality of the target theory of radiation induced effects. Essential features of non-targeted effects are that they do not require direct nuclear exposure by radiation and they are particularly significant at low doses. This new evidence suggests a need for a new paradigm in radiation biology. The new paradigm should cover both the classical (targeted) and the new non-targeted effects. New aspects include the role of cellular communication and tissue-level responses.

2.2. Low-dose effects

The cancer risk at low doses will probably never be fully elucidated by epidemiological studies, as this would require very large populations and accurate dosimetry (Brenner, et al., 2003). The dosimetry of protracted exposures is even more demanding than dosimetry for single exposures (Brenner and Sachs, 2002, 2003). Uncertainties in dosimetry of epidemiological studies make it more difficult to observe a dose response, which in turn tends to lead to lower risk estimates.

Modelling of biological events involved in radiation carcinogenesis may offer a tool to study the risk at the low dose region (Stone, 2005). The input data should contain not only the conventional direct radiation effects but also non-targeted effects which may be important modifiers of risk at the low dose region. It remains to be determined how this would apply to low-level radiation and whether it

would increase, decrease, or leave unaltered, current assessments of risk. A particular feature of non-targeted effects is the highly non-linear dose response, often downward curving at low doses, so that linear extrapolation from the high dose data would no necessarily overestimate low dose risk (Brenner, et al., 2001; Little and Wakeford, 2001). Since many non-targeted effects display highly non-linear dose response, knowledge of the underlying mechanisms is crucial to estimating low dose risks. New modelling strategies need to be employed to explore possible non-linearity in dose response resulting from complex biological models.

The genomic instability and bystander endpoints are both transmissible (mutational) and non-transmissible (lethal). The balance of these in different cellular systems may lead either to an increased or decreased risk. Some scientists indeed argue that these non-targeted radiation effects are in fact part of the adaptive response to ionising radiation and therefore protective. More research is needed on the delayed damage response systems, such as adaptive response and premature differentiation. An increase in cancer risk can be argued by amplified genomic damage, genomic instability and also by increased proliferation of cells due to cell killing. A decrease in cancer risk can be argued by cell killing removing damaged cells and adaptive response and increased differentiation of cells which may protect. During embryonic and foetal development, however, any changes altering the normal pattern of cell proliferation, cell differentiation and cell migration are likely to be harmful (Streffer, 2004).

2.3. Dose-dependency and effect of radiation quality

The dose-effect relationship for the bystander effect invariably shows a plateau below 1 Gy, and the effect appears to be determined by the dose per hit cell, rather than number of cells hit. In contrast to the targeted effects, high- and low-LET radiations appear to be equally effective in the induction of bystander response. Therefore, the bystander response appears to be an “all-or-nothing” response.

The dose-effect relationship for genomic instability shows a plateau but is a function of time at which the effects are scored, for example. High LET is more effective than low LET, but LET also influences temporal pattern of expression. The apparent difference in the RBEs of high and low LET radiations for bystander effect and genomic instability may also reflect the experimental conditions: in many of the genomic instability studies, the cell cultures contain a mixture of hit and bystander cells, whereas bystander studies, by definition, only concern non-hit cells.

Adaptive response is a biological phenomenon in which resistance to a challenging dose of radiation is established by one or several very small preceding doses. Therefore, adaptive response may be an important modifier of risk in situations where radiation exposure is protracted. Generally, and unlike most data available for genomic instability and bystander effect, the adaptive response depends on synthesis of proteins, most of which are involved in DNA damage response. Very few studies so far have tried to investigate the relationships between bystander effect, genomic instability and adaptive response. Studies on cell cultures have shown that all three effects (genomic instability, bystander effect and adaptive response) may be observed at time points distant from the initial radiation exposure (Kadhim, et al., 2004). These results extend the adaptive response to include environmentally relevant exposure situations, i.e. where the challenging dose may be far removed from an initial dose, and may affect cells that were not themselves originally irradiated.

The dose dependency for adaptive response and other non-targeted effects appears to follow a similar pattern: the adaptive response to the challenging dose does not depend much on the size of

the priming dose, but is rather a “switch on” stress response. Unlike bystander response or genomic instability, adaptive response requires protein synthesis.

Since many non-targeted effects display highly non-linear dose response, knowledge of the underlying mechanisms is crucial to estimating low dose risks. New modelling strategies need to be employed to explore possible non-linearity in dose response resulting from complex biological models.

2.4. Concept of dose as surrogate of risk

The system of radiation protection is basically built on the linear no threshold model (LNT). A linear dose response means that every increment of dose and the associated risk can be assessed separately, irrespective of prior or future doses, as long as doses are below deterministic effects, that fixed dose increment is always associated with the same additional risk, that doses received by an individual at different time points can be summed up (cumulative dose) and, collective dose can be used to predict risk at the population level.

If linearity does not hold at low doses, this would have major implications for radiation protection. Non-targeted effects challenge the LNT model and thereby the also the concept of dose as surrogate of risk.

2.5. Individual susceptibility

Individual sensitivity seems to play a role both in genomic instability and bystander effect. Animal studies indicate that some mouse strains are genetically more susceptible to genomic instability induction than others. These strains also show a higher susceptibility to radiation-induced malignancy. Genotypes that have a less effective apoptotic response seem to be more predisposed to the development of malignancy. Interindividual variation in bystander response has also been shown in human tissue. The genetic basis for this variability requires further research.

2.6. Potential mechanism for the development of diseases other than cancer

Traditionally, radiation protection regulations have been based on estimates of cancer risk at low doses and low dose rates derived by extrapolation from moderate to high dose and high dose-rate epidemiological data, in particular the Japanese atomic bomb survivors and various medically exposed groups. Recently there has been emerging evidence of risks of non-cancer health effects, in particular cardiovascular and cerebrovascular disease, in the atomic bomb survivor data and in certain medically-exposed groups (Preston, et al., 2003; Yamada, et al., 2004; Darby, et al., 2005). This is still controversial, because the shape of the non-cancer dose response in all groups, in particular the atomic bomb survivor data is not clear, and the effects have not been observed in a number of other exposed groups.

It appears likely that radiation-induced genomic instability may be linked with certain inflammatory responses (Lorimore and Wright, 2003). Although the mechanisms of non-cancer health effects are as yet poorly understood, it is very likely that inflammation is involved, particularly in relation to cardiovascular and cerebrovascular disease (Basavaraju and Easterly, 2002; Libby, 2002). This would imply a role for a non-targeted radiation effect in these disease endpoints. Since many non-targeted effects display highly non-linear dose response, knowledge of the underlying mechanisms is crucial to estimating low dose risks.

Since non-targeted cellular responses to radiation are the products of cell signalling which result in modulation of a variety of genes, including those that produce free radical scavengers and enzymes to repair DNA damage, it is expected that such exposures could impact on the risk of non-cancer effects as well as on the risk of cancer. Research to date indicates that both of these cellular responses show an "all or nothing" type of response to dose, suggesting that the first track of radiation produces the maximum gene response. If this is so, then the radiation protection concept of an effect that is proportional to dose is inaccurate at low doses, and this difficulty may apply equally to non-cancer and cancer endpoints.

3. Summary: Potential implications of non-targeted effects from the policy point of view

The observations on non-targeted effects have raised three key questions that are important from the policy point of view. First of all, non-targeted effects may modify the cancer risk in the low dose area and therefore imply a deviation from LNT at low doses. Since many non-targeted effects display highly non-linear dose response, knowledge of the underlying mechanisms is crucial to estimating low dose risks. Secondly, as it has now been shown that DNA is not the only target for radiation effects, that there are effects in non-hit cells and that the non-targeted effects may amplify radiation responses over a tissue, a shift in paradigm underlying the radiation induced health effects is warranted. It is now relevant to ask if ionising radiation may also cause non-cancer diseases or modify their risk at low and intermediate doses. The third question relates to differences in the radiation sensitivity between individuals and this issue is very relevant both for targeted and non-targeted effects.

Potential policy implications, in case there would be significant deviation from LNT (and additional detriment by non-cancer diseases) could be i.e. that the conceptual basis of the present system would be undermined, the use of dose as surrogate of risk would be seriously challenged, and the relevance of dose and the target at risk should be re-examined.

However, we should keep in mind that there is a plenty of radiobiological and epidemiological evidence that is in line with the classical paradigm. Therefore, it is advisable to build on the existing knowledge, but to see what new is brought about with the non-targeted effects. The new paradigm needs to cover both the classical (targeted) and the new non-targeted effects. New aspects include the role of cellular communication and tissue-level responses.

Acknowledgement
I am grateful to Dr. Oleg V. Belyakov (STUK) for his valuable comments.

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