

DOE Program – Developing a Scientific Basis for Responses to Low-Dose Exposures: Impact on Dose-Response Relationships

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Abstract—*The DOE Low Dose Radiation Research Program focuses on the biological mechanisms involved in the response of molecules, cells and tissues to low doses of both low and high-LET radiation (< 0.1Gy). This research program represents a merging of new technologies with cutting edge biological techniques associated with genomics. This merger makes it possible to observe radiation-induced cellular and molecular changes previously undetectable. These observed low-dose responses not only define mechanisms of interaction of radiation with living systems, but are helping characterize the shape of dose-response relationships in the low-dose region. The research from this program is suggesting a new set of radiation paradigms regarding the involvement of radiation in the carcinogenic process. This manuscript will discuss the biological phenomena observed at low doses and speculate on how these may impact the shape of dose-response relationships in the low dose region. The phenomena include initial radiation-induced DNA damage and repair, changes in gene expression, adaptive responses and bystander effects. However, this information from the cellular-molecular level cannot be directly extrapolated to risks in human populations. This extrapolation must carefully take into consideration all levels of biological organization. Links must be developed between dose-response relationships at the cell and tissue levels and risk to human populations. The challenge and the ultimate goal of the Program is to determine if basic scientific data can be combined with more traditional epidemiological methods to improve the estimation of radiation risk from low level radiation exposures.*

I. BACKGROUND

At the present time, estimation of risk from ionizing radiation is based on a linear-no-threshold extrapolation of results derived following exposure to high radiation doses. These estimates are used to predict risks at doses where changes in the frequency of diseases cannot be detected. This linear-no-threshold model has been carefully reviewed, is easy to use, and easy for the public to understand. The NCRP recently concluded that there was not adequate reason for changing the LNTH as a means of estimation of risk.¹ However, shape of the dose-response relationships at low doses needs to be further evaluated, the mechanisms behind any variations from linearity studied and the LNT hypothesis continually re-evaluated.

Following low-dose radiation exposure, the size of the exposed population required to detect an increase in the number of excess cancers is very large² making epidemiology studies in this region impossible. Thus, it becomes necessary to use the predicted number of cancers derived by linear-no-threshold calculations rather than any observed increase in cancer frequency to determine if exposures result in an increased cancer risk. This discussion highlights the need for a better understanding of the shape of dose-response relationships associated with

very low doses of ionizing radiation. In the research being funded by the Low Dose Radiation Research Program, it is possible to make measurements following low doses of radiation exposure, to determine the genes involved in the physiological and biochemical pathways associated with the biological changes and determine potential mechanisms involved in the low dose responses. Models can thus be developed that will determine if there are non-linear dose-responses and what biological processes result in such responses. This information will be useful in extrapolation from the region where cancer effects are observed to the dose and dose-rate regions where it is not possible to measure effects. It will pave the way for developing molecular epidemiological methods and in the future, supplement the standard epidemiological methods with modern biology.

As we move through the different levels of biological organization, it is possible to point to both technological and experimental advances that make it possible to understand the meaning of measurements made after exposure to low doses of radiation. This paper is organized to discuss the shape of dose-response relationships at different levels of biological organization, i.e. molecular, organelle, cellular, tissue and whole animals and the technological advances that have made it possible to study

changes induced by low doses of radiation. It will speculate on the influence of new biology on the shape of dose-response relationships in the low dose region and evaluate the need for new paradigms on how radiation interactions with biological systems.

II. LINEAR PROCESSES

Deposition of Energy

Energy deposition events after exposure to ionizing radiation are randomly distributed in the tissue so that the initial interaction of radiation with cells and molecules represent a random or stochastic process. The number of interactions increases linearly with increased dose. It is the total number of interactions, the distribution of these interactions and the time-related frequency of the energy depositing events that are responsible for the biological changes observed. The challenge is to determine the relationship between observed biological change and change in risk for development of an adverse health outcome. Thus, it is necessary to evaluate each radiation-induced biological change to determine if it has a potential impact on the risk of developing radiation-induced cancer.

DNA Damage

Initial radiation-induced DNA damage following high doses of high-LET radiation was demonstrated to increase as a linear function of dose and energy deposition.³ New techniques have demonstrated that even for lower doses, radiation-induced DNA damage seems to be linearly related to dose.⁴ In addition, it has been possible to visualize the induction of damage in individual cells using labeled protein foci that are thought to be formed at the site of the DNA damage.⁴ This use of the γ H2AX protein has been related to the induction of DNA damage and shown to increase linearly with dose down to very low levels of exposure.⁵ This study also demonstrated that the loss of γ H2AX foci did not occur following low doses of radiation. This lack of repair and linear increase as a function of dose supports the concept that the deposition of the energy and the initial radiation-induced DNA damage increases linearly with dose even following low levels of exposure.

If DNA damage and repair of that damage are unique for radiation, then each unit of damage may be considered to represent an increase in risk resulting in linear dose-response functions. Methods have been developed that make it possible to carefully measure the number and types of radiation-induced damage and the localized distribution of the damaged sites.⁶ This research characterizes the distribution of DNA damage sites induced by normal endogenous processes compared to radiation-induced DNA damage.^{6,7,8,9} This research suggests that radiation produces sites on the DNA where there are multiple

damages in a small area. This radiation-induced DNA damage is different from damage produced by normal oxidative stress in cells. The repair of these locally damaged sites is the subject of continuing research.

Gene Expression

Microchip technology has made it possible to rapidly measure changes in gene expression. With microchip methods, the changes in the level of gene expression in thousands of genes can be measured at one time. It is also possible to develop specialized gene chips that focus on genes involved in different well-defined biological processes such as the induction of DNA repair^{10,11,12,13} or apoptosis.¹⁴ Studies have been conducted to determine the genes involved in the biological responses elicited by exposure to graded radiation doses.^{15,16} For certain genes, both a dose and a time dependent change in response after exposure were observed. For selected genes, the dose-response relationship was linear down to doses as low as 0.02 Gy.

III. NON-LINEAR PROCESSES AND THRESHOLDS

In spite of the recommendations associated with the policy and regulatory use of the Linear No Threshold Hypothesis (LNTH), it is important to recognize that there are many data sets and biological processes that do not support it. These data suggest that many radiation-induced biological processes involved in cancer induction are non-linear and that energy, biological, practical and statistical thresholds exist. Such thresholds must be considered in evaluation of risk and support non-linear functions for radiation-induced cancer.

Energy Barriers

One of the factors that support the LNTH is the fact that many experimental systems show linear-dose-response relationships. In many experiments, dose-response relationships are derived and continue down to doses where the response is no longer significantly different from the background response. Often when this non-significant dose point is reached, investigators repeat the experiment with more subjects. By adding subjects to the low dose groups, they demonstrate that a data point that was not significant under the first set of experimental conditions can be made to be significant in the second study. It is, however, the total energy deposited in the biological system under study or the number of energy deposition events that produces the biological response, not the energy concentration. If this is true then the argument can be made that the proper unit to plot on the X-axis as the variable that produces the biological change on the Y-axis should be total energy deposited in the system and not dose. It is also important to note that energy can be added

and that dose, as a ratio of energy per unit of mass is non-additive. Energy is the proper metric to be related to another additive quantity, the net excess responses relative to the background response. These two metrics are both additive and can be summed across the number of subjects in the study. When energy is used in the above example, the addition of subjects to the low exposure group also adds energy to that part of the experimental system and moves the energy metric on the X-axis to a larger value. This unmasks the property of dose that makes it possible to postulate the LNTH. With the use of energy, it is easily demonstrated that there is an energy level for most biological endpoints below which it is not possible to demonstrate an increase in biological response.¹⁷ This creates energy barriers below which significant biological responses cannot be measured. This barrier can be demonstrated for any biological endpoint where both the background response and the sensitivity of the biological system to radiation-induced changes can be defined.

Molecular and Cellular Thresholds

1. DNA Repair

There is extensive research directed toward understanding the nature of initial DNA lesions. This understanding helps determine if endogenous DNA damage that is produced by reactive oxygen species in many normal physiological processes is similar or different from that produced by radiation. Normal body functions produce large amounts of DNA damage that is eliminated by error free homologous recombinational repair.¹⁸ If repair of radiation-induced DNA damage and the damage induced by normal endogenous cellular and molecular processes are similar at low doses, there could be non-linear threshold types of responses below which the normal processes repair the DNA damage in an error free way. Error free DNA non-homologous end-joining repair (NHEJ) has been postulated to repair most of the DNA damage induced by endogenous factors. If this type of repair also can correct radiation-induced DNA damage after low doses, it may produce a biological threshold. As the dose increases, the amount of DNA damage may reach a level where there are not enough enzymes responsible for the DNA repair to properly correct it. At these doses, the DNA damage could act as a signal to trigger other biological responses, and may progress to result in increased levels of mutations or chromosome aberrations above the normal background. The ability to repair DNA damage at low doses could be thought of as a biological threshold.

2. Gene Expression

Research conducted at Lawrence Livermore National Laboratory using human cells determined that the spectrum

of genes that respond by changing their level of expression following radiation exposure is dose dependent. The number and type of genes that are activated by low and high doses of radiation have been evaluated. At INIH, it was demonstrated that there is a different set of genes up-regulated by high doses of radiation than were altered by low doses.^{19,20} In the studies at Livermore, it was possible to determine that there is a "break point" between the induction of different sets of genes. At doses below about 10-15 cGy, "low dose genes" are activated and at doses above this level of exposure, another set of "high dose genes" are activated. These genes are being characterized and suggest that it is not possible to extrapolate the genetic response observed following high doses of radiation to the changes in gene expression observed after low doses. There were relatively few genes responding to both high and low doses of radiation. The genes involved in low doses of radiation may be responsible for a different set of biological processes than those stress genes that are activated after high doses of radiation. This change of gene expression could also be thought of as a threshold where one set of responses stops and a second set starts.

3. Adaptive Response

One response that may be associated with these low-dose-induced gene changes is the adaptive response. Adaptive responses are present following low doses of low-LET ionizing radiation. The presence of an adaptive response is now widely accepted by the radiation research community.

The adaptive response is defined in two different ways. First, it is defined as a reduction in responsiveness to a large challenge dose induced by a previous low radiation adaptive dose.²¹ Second, the adaptive response has been defined as using a low dose of radiation to decrease the spontaneous or background level of cancer or other biological endpoint.^{22,23} A large number of studies were reviewed that demonstrate an adaptive response.²⁴ It is important to determine if an adaptive response can decrease the cancer risk from radiation exposure at low levels. It has been suggested that the low doses of radiation produce a protective response for the induction of leukemia and osteosarcoma in mice.^{22,23} Using gamma rays, it has also been determined that low doses of radiation (less than 0.10 Gy) decrease the number of transformed human cells to values that are lower than observed in the control cells.^{22,26,27} As the dose is further increased, the frequency of transformation increases. These examples suggest that risk from radiation at low doses may be less than that predicted from the linear-no-threshold model.

4. Bystander Effects

A major technological advance being developed and used in the DOE Low Dose Program is the development of microbeams. With a microbeam it is possible to expose cells to alpha particles,^{28,29} protons or electrons,³⁰ and focused low energy x-rays.³¹ The impact of the microbeam on the field of radiation research has been carefully reviewed.³²

After exposing individual cells and parts of cells to radiation from the microbeam,^{29,30} modern cellular and molecular techniques are used to study the changes that occur in the "hit" cell as well as in neighboring cells that were not directly traversed by the radiation. It has been possible to demonstrate that the "hit" cell communicates with its neighboring cells and triggers cellular and molecular changes in these cells.^{33,34,35} This has been termed the "bystander effect".

With this equipment, it is possible to study the role of the bystander effects on the induction of mutations when only the cytoplasm was exposed to the radiation.³⁶ It has also been possible to determine that biological changes such as chromosome damage^{24,37,38} and cell transformation^{33,34,39} can be induced in "bystander" cells that do not have energy directly deposited in them. With such equipment, the concept of single cell "hits" as they relate to damage and to dose becomes challenged.

Research has investigated the shape of the hit number-response relationship for the induction of cell transformation. Cell transformation is thought to be an early step in the conversion of normal cells to cancer cells and provides an early indication of increased risk. Studies were conducted that relate the frequency of cell transformation to the radiation dose and the number and distribution of alpha particles in a cell population. It was been demonstrated that giving each and every cell one and only one alpha particle is not as effective in producing cancer as giving the cells an average of one alpha particle.³⁴ It was also determined that delivering equal numbers of alpha particles to all the cells or to only 1 in 10 cells resulted in the same number of cell transformations.³⁵

These responses have all been observed in tissue culture with the cells grown in mono-layer. This unnatural physiological state may have a marked effect on the way the cells respond to radiation insult. For the bystander effect to be of significance in terms of risk assessment, it is important to determine if these effects are produced *in vivo* using experimental animals, and finally if they are present in humans.

Experimental animal studies using injected ²³⁹Pu oxide particles illustrated that the bystander effect may be present in Chinese hamster liver.^{40,41} Chinese hamsters were injected with three different ²³⁹Pu oxide particles particle sizes. The classic radiobiology or "hit" theory predicted that there would be a very large response following exposure to the alpha particles from the small particles and a small response following local dose from the large particles. However, in this study the number of

chromosome aberrations⁴⁰ and cancers⁴¹ increased as a function of total dose to the liver, and not as a function of local dose to hit cells, number of alpha traversals per cell, or number of cells traversed by alpha particles. These data suggest that the liver was responding to the insult from the plutonium as an organ. The cells hit with large amounts of energy are capable of signaling the non-hit cells to result in the same amount of damage per unit of energy deposited in the organ. Thus, bystander effects are demonstrated for alpha particle exposure both in tissue culture and in experimental animals.

The cellular signaling involved in the "bystander" effect and its role in carcinogenesis has been reviewed.⁴² Extra-cellular signaling integrates multi-cellular damage responses that are important deterrents to the development of cancer through mechanisms that eliminate abnormal cells and inhibit neoplastic behavior. The role of the extra-cellular matrix and stroma on radiation-induced cancer have been reviewed.⁴³ These observations suggest that perhaps the bystander effects could, in some cases cause, damage in non-hit cells and increase risk, while in other cases, it may produce signals that are protective and produce non-linear dose-response relationships.

It is critical to determine if "bystander" responses increase or decrease the risk for production of late occurring disease. The current extrapolation of bystander studies to suggest changes in human cancer risk is premature and requires additional research.

IV. COMBINING BIOLOGICAL PHENOMENA TO EXPLAIN DOSE-RESPONSE CURVES

To provide an example of how different biological processes influence the shape of dose-response relationships, the induction of an endpoint thought to be important in cancer induction, that is chromosome aberrations, has been evaluated.^{44,45} The shape of the dose-response relationships for the induction of chromosome aberrations has been carefully defined and has been used extensively in biodosimetry. It has been known for a long time that exposure of cells to high-LET radiation like alpha particles from radon or ²³⁹Pu results in linear relationships between dose and the frequency of aberrations/cell. It is also well established that there are non-linear dose-response relationships between the induction of chromosome aberrations and exposure to high dose-rates from low-LET radiation.⁴⁶ An example of a typical dose-response relationship between the induction of chromosome aberrations and the type of radiation exposure has been published.⁴⁶ Is it possible to examine the molecular mechanisms that result in these very different dose-response relationships as a function of exposure type? In the past these curves have been explained based on the "hit" theory. That is the energy deposited in a cell following the traversal of a single high-LET alpha particle was thought to produce enough damage to directly induce

a chromosome aberration. For low-LET radiation it was postulated that multiple "hits" were required to deposit enough energy in the nucleus of a cell to produce exchange type chromosome aberration. In addition to the direct action of the low-LET radiation, some aberrations were postulated to be produced by indirect actions of radiation. This interpretation has been supported by a large number of studies that evaluated the difference between high- and low-LET radiation and the ability of each of these to produce direct and indirect effects. These studies were carefully reviewed⁴⁷ and the conclusions from this review seem to have stood the test of time.

However, with the advent of recent studies on radiation-induced chromosome damage associated with both the adaptive response and bystander effects, it is possible to evoke a new radiation paradigm to explain the shape of these dose-response relationships. It can be postulated that the linear dose-response relationship observed for the induction of chromosome aberrations following exposure to high-LET radiation is a combination of bystander and the direct effects. It has been demonstrated that after traversal of a single alpha particle through a single cell, chromosome damage can be produced in both the cell that is "hit" by the alpha particle and in "bystander cells" with no energy deposition.³⁸ This bystander effect results in the low dose induction of the chromosome aberrations. As the dose increases, the frequency of bystander effects remains constant³⁸ and the frequency of directly induced aberrations continues to increase. The combination of these two processes, bystander and direct effects could result in the apparent linear increase in chromosome aberrations even for very low doses from alpha particles.

The alternative explanation for the non-linear dose-response observed following exposure to low-LET radiation could be a combination of the adaptive response that decreases the number of directly produced chromosome aberrations and the fact that bystander effects have not been demonstrated following very low doses of low-LET radiation. At low doses, the adaptive response prevents the formation of aberrations. As the dose increases, the number of aberrations produced by direct effects increases as well as the potential for deposition of enough energy in the nucleus to induce bystander effects, which also produce aberrations. This could explain the non-linear shape of the dose-response relationship at low doses.

Again the differences in the shape of dose-response relationships may be related to the fact that high-LET radiation is very effective in producing the bystander effects and not effective in producing of adaptive responses. This results in linear dose-response relationships at low total doses of high-LET radiation. On the other hand, low doses of low-LET radiation are very effective in producing protective adaptive responses and not capable of producing the bystander effect. This

combination results in non-linear dose-response relationships at low total doses.

As research is conducted to define the genes and signals involved in both the adaptive response and bystander effects, this hypothesis can be directly tested. Such tests may help explain the shape of the dose-response relationships for this cellular endpoint following exposure to very low doses of ionizing radiation. It is of interest to determine if such responses at the cellular level can play a role in providing an explanation for mechanisms involved in radiation-induced cancer.

V. CANCER INDUCTION

Linear Relationships

It is well established that there are both linear² and non-linear⁴⁸ dose-responses for radiation-induced cancer in both humans and experimental animals.^{49,50} The data that is the most widely used to set radiation standards is from the A-bomb survivors.^{51,52} Preston suggests that, "Excess solid cancer risks appear to be linear in dose even for doses in the 0-150 mSv range".⁵² Extensive evaluation of these data by the NCRP¹ and others² recommend that linear no threshold dose-response relationships be used in making policy related to radiation protection as a conservative estimate of radiation risk.

It seems that initial interaction of the radiation with matter and the initial damage produced is linear. The suggestion that these linear observations be extended across all biological changes involved in the final outcome of cancer is very difficult to justify based on the many processes involved in converting cells of a normal tissue to cancer. There are many biological pathways that are linear and others that are non-linear. For each tumor type and tissue type there are unique pathways and changes that are necessary for converting normal tissue into cancers.⁵³ The primary pathways involved in the development of each type of cancer will determine the shape of the dose-response relationship for that cancer.

Practical Thresholds

Early research on the induction of bone cancer in humans "radium dial painters" showed very non-linear dose-response relationships.⁵⁴ Similar non-linear responses with rather large "biological or sensitivity thresholds" have been observed for bone⁵⁵ and lung cancer⁵⁶ in many experimental animal models. There are some tissues that are very sensitive to radiation-induced damage and others that seem to be very resistant.

This difference in responsiveness seems to be important in the shape of dose-response relationships for cancer induction in experimental animal. In mice exposed to external radiation there is a unique shape of dose-response for each tumor type.^{49,50} In rats exposed to radon

and its daughter products the frequency of lung tumors in these animals was well documented and related to exposure and dose.⁵⁷ However, there was never a tracheal tumor observed in these animals, even though the dose and chromosome damage to the tracheal cells was not very different from the dose to the deep lung cells.⁵⁸ Such data suggest there is a very large threshold of exposure and dose for the induction of cancer in the trachea. These large "thresholds" are responsible for the use of tissue weighting factors in radiation protection.⁵⁹

The other type of threshold associated with animal data has been called a "practical threshold" and is associated with the radiation dose-rate from internally deposited radioactive materials. In these studies, the rate of accumulation of dose and damage could be shown to be the important variable in the induction of cancer from internally deposited radium and strontium. At low dose rates, the total dose and damage accumulated over the life time of the experimental animals was not high enough to get a significant change in the frequency of cancers induced by the treatment. Thus, the animals died before they could accumulate an insult that was high enough to trigger a cancer response. These data have been carefully evaluated as a function of both dose and dose-rate. It was determined that practical thresholds exist for bone cancer induction at very large doses, below about 500 rem (5.0 Sv) (using a quality factor of 10) for dogs, mice and people.⁶⁰ This provides further support for the hypothesis that large radiation doses and dose-rates are required to produce many types of sarcoma. The criticism of these data is centered on the small size of the experimental population and the suggestion that if the population size would have been much larger there would have been a significant response at lower dose-rates. Such information further supports the postulate that there are energy thresholds below which it is not possible to observe a response. By adding additional subjects at lower dose rates, one would of course, be adding energy to get a significant response.

This section illustrates that the complex biology of cancer induction plays an important role in the shape of dose-response relationships. There are both linear and non-linear processes involved and, depending on the biological endpoint measured, the tumor type, tissue type and exposure, the shape of the dose-response relationship can be driven by either a linear or non-linear process. It is important to consider all these linear and non-linear processes in making risk estimates.

VI. CONCLUSIONS

The research funded by the DOE Low Dose Radiation Research Program has provided new information suggesting that basic radiation paradigms may need re-evaluation. From this paper, it can be seen that there are processes involved in radiation-induced biological changes

that are both linear and non-linear. The initial energy deposition events and the induction of some molecular changes seem to be linear. The repair and processing of the damage, on the other hand, has some very non-linear components. Examples of these have been discussed.

The adaptive response suggests that cells recognize low doses of radiation and change gene expression. These changes result in alterations of normal processes and may provide a protective effect against either background damage or subsequent radiation exposure. The genes involved in this response are being evaluated and with the understanding gained, it may be possible to determine how these activated genes may protect against late effects of very low doses of ionizing radiation.²⁵

In the past, it has been assumed that a cell has to have energy deposited in it to produce a response from radiation exposure. The observation of "bystander effect" demonstrates that this is not the case. Such phenomenon requires a re-evaluation of how dose-response relationships are constructed and what metrics are proper to use in this reconstruction. The wide range of change in radiation-induced gene expression also casts some doubt on the mutation theory of cancer and suggest that changes in gene expression can also change cell phenotype and transformation.⁴² Cell/cell communication and the total tissue responses to radiation suggest that the cellular and matrix environment following radiation may play a large role in the development of disease.⁶¹

This paper provides a quick review of the current thinking on how some of these new biological observations may impact the shape of the dose-response relationships for radiation-induced disease. Of course, at this point, early biological endpoints are not directly linked to disease and require additional research. If the early endpoints were linked to disease, as suggested with the example for chromosome aberrations, it would seem that the adaptive response could result in a sub-linear dose-response for the risk from exposure to low-LET radiation. Bystander effects would have little influence on the shape of the dose-response relationships for risk from low-LET radiation, but could result in either a linear dose-response or even super-linear response in risk to high-LET radiation delivered at very low doses.

Current efforts are being made to insure that this newly developed information is used in development of appropriate models to predict radiation risk. An important part of this program is to disseminate this new information. A web site has been developed for this purpose and can be found at <http://lowdose.tricity.wsu.edu> to ensure open communication of the results of this program to the public as well as to the scientific community. The final hope is that the research will decrease the uncertainty associated with the level of risk for induction of radiation-related disease. With this new mechanistic understanding, the rational for radiation protection will be strengthened.

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