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LOW DOSES OF RADIATION REDUCE RISK *IN VIVO*

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□ The "Linear No Threshold" hypothesis, used in all radiation protection practices, assumes that all doses, no matter how low, increase the risk of cancer, birth defects and heritable mutations. In vitro cell based experiments show adaptive processes in response to low doses and dose rates of low LET radiation, and do not support the hypothesis. This talk will present cellular data and data from animal experiments that test the hypothesis in vivo for cancer risk. The data show that a single, low, whole body dose (less than about 100 mGy) of low LET radiation, given at low dose rate, increased cancer latency and consequently reduced both spontaneous and radiation-induced cancer risk in both genetically normal and cancer-prone mice. This adaptive response lasted for the entire lifespan of all the animals that developed these tumors, and effectively restored a portion of the life that would have been lost due to the cancer in the absence of the low dose. Overall, the results demonstrate that the assumption of a linear increase in risk with increasing dose in vivo is not warranted, and that low doses actually reduce risk.

I. INTRODUCTION

All current radiation risk estimates and all radiation-protection standards and practices are based on the so-called "Linear No-Threshold Hypothesis". This LNT hypothesis is in turn, based mainly on epidemiological data of humans exposed to high doses and dose rates but is considered to also apply at low doses and dose rates, with a two-fold reduction in risk. The hypothesis states that:

1. Risk per unit dose is constant without a threshold.
2. Risk is additive.
3. Biological variables are insignificant compared to dose.

These assumptions allow radiation dose to be used as a surrogate for radiation risk. However, at low doses the LNT hypothesis is acknowledged to be an assumption, and other dose responses are also possible, including supralinear, sublinear or threshold/hormetic responses. This paper presents data testing the validity of low dose risk estimates that are based on the LNT hypothesis, and will focus on cancer risk, considered to be the most important measure of risk. However, other papers presented at this meeting will focus on teratogenic effects and heritable mutations, also important measures of risk.

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While ultimately the influence of low doses on the risk of carcinogenesis must be measured *in vivo*, it is important to understand the mechanisms underlying any such effects. Experiments conducted in other organisms, or based on human and other cells grown in issue culture can provide such information. Therefore, in addition to *in vivo* data, this paper also presents the results of cellular experiments that indicate the mechanisms that may be involved.

If we consider the potential biological consequences of a radiation exposure to a normal cell, there are three general biological outcomes of DNA damage[1] as shown in Figure 1. When DNA damage is created as a result of one or more tracks of radiation through a normal cell, the cell will attempt to repair that damage. If the repair is successful and the DNA restored to its original state, i.e., an error-free repair, then the cell is also restored to normal. In this case, there is no resulting consequence to the cell and hence no resulting risk. Another possibility is that the cell recognizes that it cannot properly repair the damage, and as a consequence activates its genetically encoded cell death process, called apoptosis. Again, in this case, no risk of carcinogenesis results since dead cells do not produce cancer. The third possible outcome of the DNA damage is repair that avoids cell death but which is error-prone, resulting in a mistake that creates a mutation. At this point, the cell may still activate its apoptotic cell death program but could also simply resume dividing. Creation of these errors is part of a process called genomic instability, which can ultimately lead to cancer. Of the three possible outcomes, therefore, only one creates a risk of carcinogenesis. It is useful to remember that the LNT hypothesis implies that risk is influenced only by dose, and hence predicts that the relative proportions of these three biological possibilities must be constant. If they were not constant, then risk would vary with their relative proportions, and not strictly as a function of dose.

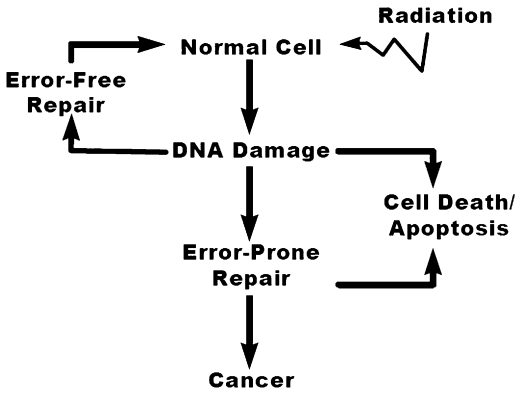


FIGURE 1. Possible outcomes of a cellular radiation exposure in a normal cell

II. EXPERIMENTAL RESULTS AND DISCUSSION

Cellular Studies

A common result of a radiation exposure in cells, particularly a high dose exposure, is a break in one or more chromosomes, which indicate DNA double strand breaks. If cells divide before repairing those breaks, the remaining pieces of chromosomes are packaged into micronuclei (MN). Measuring the frequency of MN in cells that have been exposed and allowed to repair therefore represents a measure of the competence of the cells at repairing such chromosomal breaks (and therefore DNA double strand breaks) in response to radiation damage. We have tested the influence of low doses and low dose rate exposures on the ability of human skin cells to repair radiation breaks in chromosomes[2]. Figure 2 shows the MN frequency in cells exposed to a variety of doses (1-500 mGy) delivered at a low dose rate (3 mGy/min) 3h before exposure to a high dose (4 Gy) delivered at a high dose rate (1.8 Gy/min). The LNT hypothesis predicts that the consequences of the two doses would be additive and yet the experiment shows that they are not. The combined exposure resulted in fewer broken chromosomes than the single acute 4 Gy exposure alone. The figure also shows that enhanced repair occurs after 1 mGy, the lowest γ dose possible in a single cell since it represents, on average, a single track per cell. The figure also shows that higher doses, representing multiple

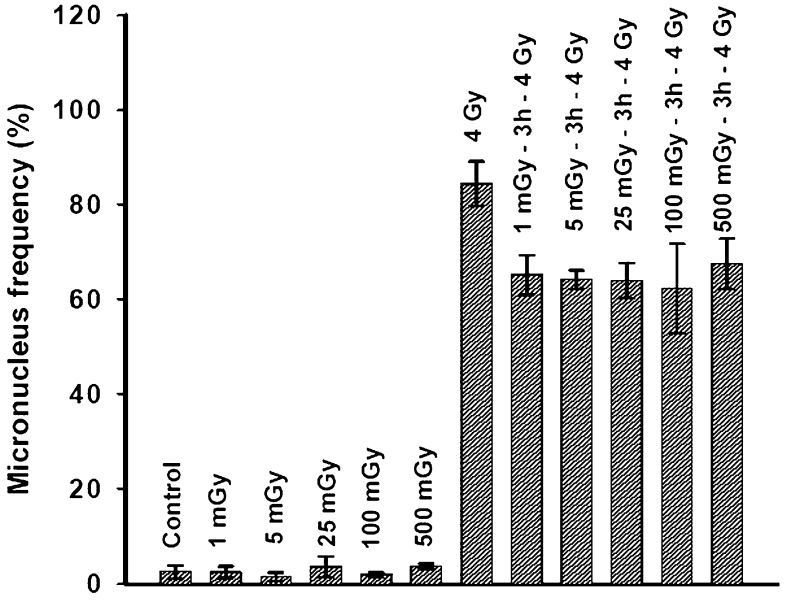


FIGURE 2. Low doses enhance the repair of broken chromosomes in human cells.

tracks/cell, produce the same result as one track/cell when those tracks from the higher doses are spaced out in time (3 mGy/min). This type of analysis can be applied to *in vivo* situations that have particular importance in the environmental assessment and licensing of nuclear installations. For example we have shown[3] that this adaptive response also occurs in fibroblasts taken from wild white tailed deer, and therefore the consequences of radioactive contamination in the environment may not be as predicted by the conventional LNT assumptions.

A direct test of this idea is shown in Figure 3, which gives some preliminary data[4] obtained from leopard frogs living in either a radiologically clean pond or in a pond contaminated with tritium and ^{14}C that gave an annual dose of about 1 mGy. Liver cells from the frogs living in the clean pond showed a normal adaptive response to low doses, as was seen in the human cells in Figure 2. However, exposure of the frogs from the contaminated pond to a large dose produced comparatively little increase in chromosomal breakage in their liver cells, and this was only slightly reduced if the frogs were given a prior low adapting dose.

This lack of chromosomal damage after a high dose indicates that the frogs were already adapted to radiation by their environmental exposure. This *in vivo* measure of the consequences of environmental radiation exposure indicates that low levels of radioactivity in the environment may not be harmful to organisms, and may only serve to enhance cellular defence mechanisms. Evidence that improvement of cellular defence mechanisms after low dose exposures is actually reducing cancer risk is

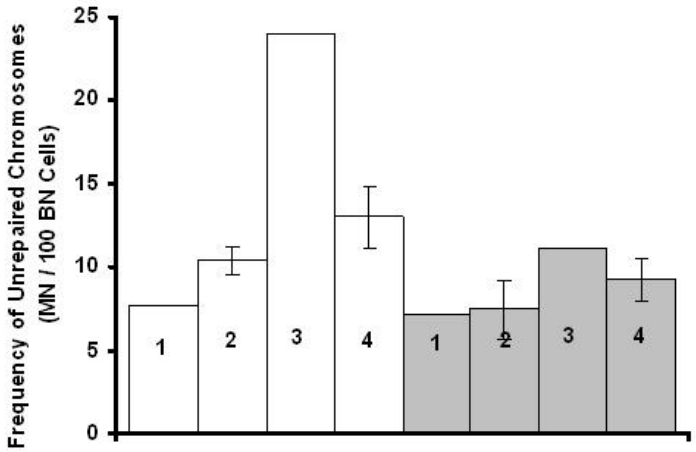


FIGURE 3. Repair of chromosome breaks in liver cells taken from frogs living in an uncontaminated pond (open bars) or a pond contaminated with ^{3}H and ^{14}C (shaded bars) delivering about 1 mGy/y to the frogs. 1. No further radiation exposure. 2. Frogs receiving 1-100 mGy of ^{60}Co gamma radiation at low dose rate. 3. Frogs receiving 4 Gy of ^{60}Co gamma radiation at high dose rate. 4. Frogs receiving 1-100 mGy at low dose rate 3h before 4 Gy at high dose rate.

TABLE 1.

Treatment	Transformation Frequency ($\times 10^{-4}$)
Control	3.7
4 Gy (high dose rate)	41
100 mGy (low dose rate) + 24h + 4 Gy (high dose rate)	16

shown in Tables 1 and 2. Table 1 shows the results of an experiment[5] using rodent cells and examining the influence of a prior low dose, given at low dose rate, on the risk of malignant transformation resulting from a subsequent high radiation dose. The risk associated with the high radiation dose was reduced by a factor of 2-3 by the prior low dose, a result that parallels the evidence for improved repair of radiation damage shown in Figure. 2.

The ability of a low dose to reduce the risk of a subsequent high dose has importance for medical types of exposures, such as those used in cancer therapy, and for estimates of environmental impact for the nuclear industry. However, the effect of the low dose alone is of more importance for human exposures in the nuclear industry. Table 2 shows the experiment testing the effects of low doses alone on malignant transformation in rodent cells[6]. All the doses tested, between 1 and 100 mGy, given at low dose rate, reduced the risk of spontaneous malignant transformation, and all doses were equally effective. The lowest dose tested, 1 mGy of ^{60}Co γ radiation, represents an average of 1 ionization track per cell, the lowest dose physically possible in one cell. Since radiation tracks are random, not all cells actually receive one track, but all respond to the same extent as they did when they certainly received one or more tracks at the higher doses. This is evidence therefore, that not all cells are actually required to be exposed (hit) by radiation in order to enhance their defences and reduce their risk. Such distributed effects are known as bystander effects and result from inter-cell signalling.

Cancer Risk in Animals

While experiments in cells provide important supporting information about the actual molecular and cellular responses to low doses, ultimate-

TABLE 2.

Treatment	Transformation Frequency ($\times 10^{-3}$)
Control	1.8
1 mGy	0.53
10 mGy	0.42
100 mGy	0.53

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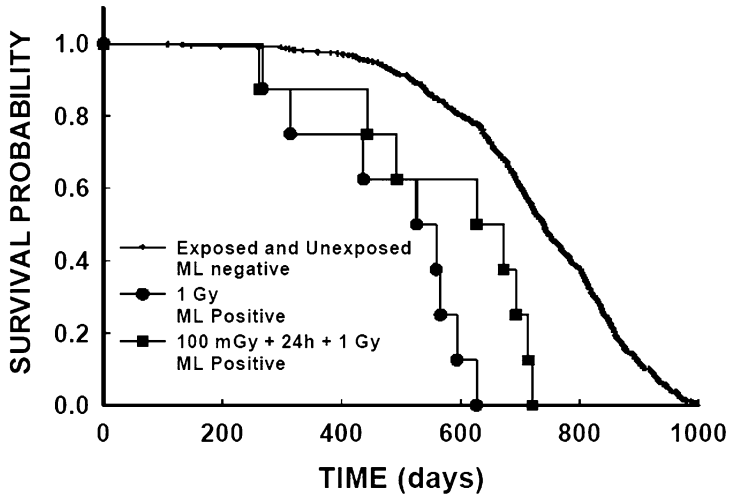


FIGURE 4. Delayed appearance ($P < 0.001$) of radiation-induced myeloid leukemia (ML) in genetically normal mice by exposure to 100 mGy at low dose rate 24h before the carcinogenic 1 Gy exposure.

ly experiments testing the effect of low doses on measures of risk such as cancer must be conducted in whole mammals. Figure 4 show a test[7] of the influence of a low dose exposure on radiation-induced myeloid leukemia in genetically normal mice. The figure shows that exposure to a high dose of 1 Gy induces myeloid leukemia in mice, and as a result, those mice with the disease lose a substantial portion of their normal lifespan. However, increasing the total exposure, by exposing the mice to 100 mGy, at low dose rate, the day before the 1 Gy exposure delayed the onset of those cancers ($P<10^{-3}$), effectively restoring a portion of the lifespan that would otherwise have been lost in those mice that developed the disease. It is important to note that the low "adapting" exposure did not affect the frequency of the disease induced by the high radiation dose, only the latency. The carcinogenic process is thought to involve an initiating event, which subsequently triggers an accelerating process of genomic instability leading to multiple genomic rearrangements, ultimately producing a cancer cell. The frequency of cancer is thought to reflect the number of initiating events while the latency of the disease reflects the rate at which the genomic instability process proceeds. The results shown in Figure 4 indicate that low doses delivered at low dose rates slows the rate of progression of the genomic instability process but does not change the frequency of the cancer initiating events[7].

Radiation protection standards and practices applied to humans must consider the possibility that some individuals may be radiation-sensitive and cancer-prone for genetic reasons. This raises the possibility that low doses may produce effects in such individuals that are different, and potentially more harmful, than those seen in genetically normal individu-

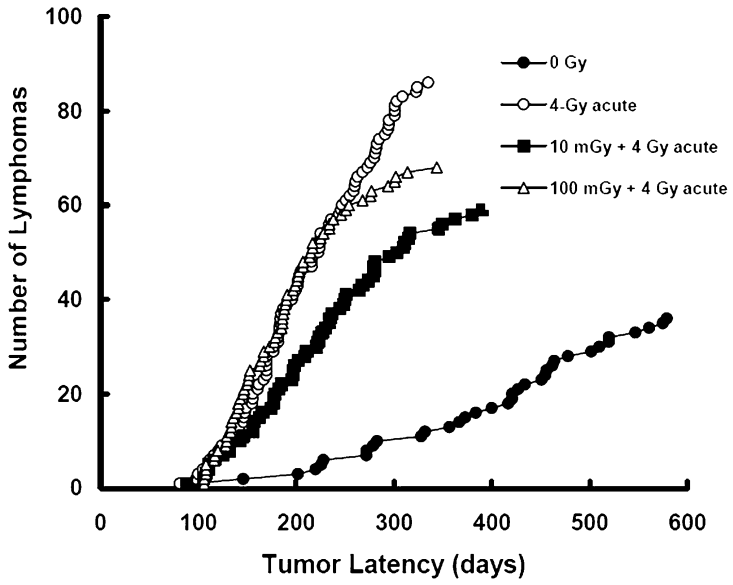


FIGURE 5. Appearance of lymphomas in unexposed cancer prone mice (Trp53 +/-) and in mice exposed to 4 Gy with or without a prior low dose and dose rate exposure.

als. Figure 5 shows a test[8] of this "worst case scenario". Mice that are heterozygous for the p53 gene (Trp53 +/-) are compromised in their ability to repair DNA damage and in their ability to initiate cell death in improperly repaired cells (Figure 1). Consequently, such mice are both cancer prone and radiation sensitive. Figure 5 shows that these mice spontaneously develop lymphomas, and a high 4 Gy dose of radiation increases the frequency and dramatically accelerates the appearance of these tumours. A dose of 10 mGy, given at a low dose rate the day before the 4 Gy exposure, delayed the onset of these lymphomas ($P<10^{-4}$), but did not significantly change the frequency. Correcting for competing causes of death did not change this conclusion. This effect of increasing latency was also seen in the genetically normal mice in Figure 4. Increasing the low adapting dose to 100 mGy caused this protective effect to disappear. While not increasing harm, 100 mGy apparently represents an upper threshold for doses that are protective against radiation-induced lymphomas.

Experiments testing the *in vivo* effect of low doses on cancer risk produced by high dose exposure are important for improving our understanding of the dominant biological outcome of such exposures, and are potentially useful concepts for medical radiotherapy procedures. However, for radiation protection standards and practices in the nuclear industry, it is more important to understand the influence of low doses on spontaneous cancer risk. Figure 6 shows the results of such a test[9] in

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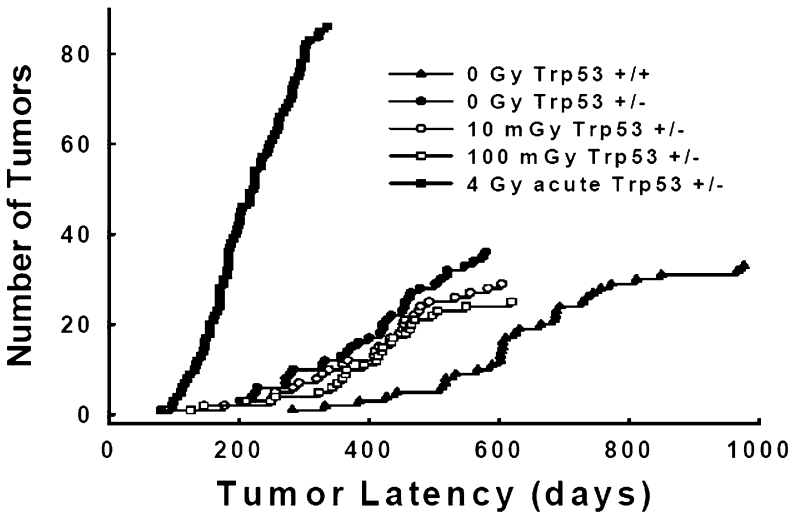


FIGURE 6. The appearance of lymphomas in unexposed or radiation exposed genetically normal (Trp53 +/+) or cancer prone (Trp53 +/-) mice.

the cancer prone p53 heterozygous mice that represent the "worst case scenario". The figure shows that unexposed, genetically normal mice (Trp53 +/+) of this strain also spontaneously develop lymphomas but that these cancers appear much earlier in the unexposed, cancer prone (Trp53 +/-) mice. Exposure to an acute 4 Gy dose of radiation dramatically accelerated this appearance. The figure also shows that a single exposure of either 10 or 100 mGy, given at low dose rate to young mice, restores a portion of the lifespan lost due to the disease in the unexposed, cancer-prone mice ($P<10^{-4}$). Unlike the result in Figure 5, where the lymphomas developed in mice that had subsequently received a high dose, the protective effect against these spontaneously appearing cancers was not lost when the dose was increased to 100 mGy. This result suggests that the upper dose threshold for protective effects varies with the severity or nature of the cancer-inducing event, with the threshold being higher for less severe inducing events such as spontaneous occurrences.

Other tumours also appear spontaneously in these cancer-prone mice. Osteosarcomas develop in the spine and grow to the point where they create paralysis in the mice. Figure 7 shows the time that these spontaneous cancers create paralysis in the mice, with and without a single exposure to 10 mGy given at low dose rate when the mice were 8 weeks old[8]. Compared to the mice not receiving the low dose, the appearance of the first spinal osteosarcoma was delayed by more than 100 days, and that delay persisted for all of the tumours that appeared, i.e. for the entire lifespan of the mice (P=0.005). This lifetime protection was also apparent

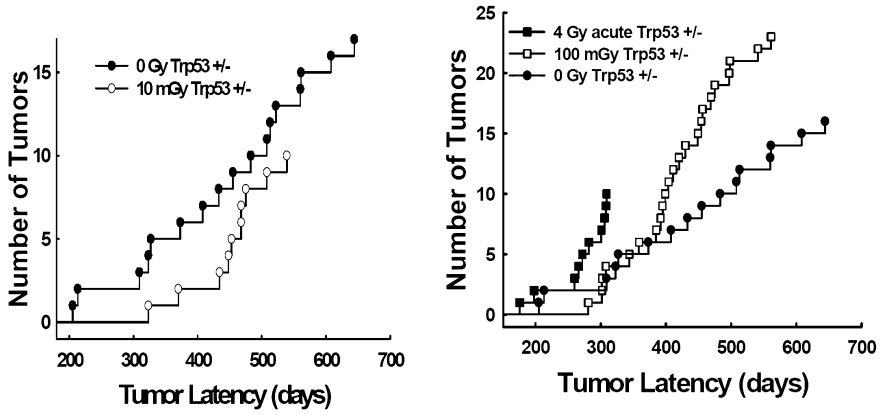


FIGURE 7. The appearance of spontaneous osteosarcomas in unexposed cancer prone mice (Trp53 +/-), or exposed to 10 or 100 mGy at low dose rate. Exposure to 4 Gy is shown for comparison.

for the spontaneous lymphomas shown in Figure 6. However, unlike the case for spontaneous lymphomas (Figure 6), increasing the low dose to 100 mGy resulted in a general acceleration of the appearance of the spontaneous spinal osteosarcomas ($P<0.04$, Figure 7). This decrease in cancer latency clearly represents an increase in risk, rather than the risk decrease seen at 10 mGy. For this tissue type therefore, the upper threshold for protective effects of a low dose must lie between 10 and 100 mGy. Since, in the same animals, the upper dose threshold for protection against lymphomas exceeded 100 mGy, we conclude that the dose threshold, where protective effects give way to detrimental effects, is tissue-type specific.

III. CONCLUSIONS

This paper has described experimental tests, at the molecular, cellular and whole animal levels, of the validity of the Linear No-Threshold Hypothesis at low doses and dose rates. Using a variety of endpoints, some surrogate for risk estimates and others direct measures of cancer risk *in vivo*, the hypothesis has failed at all levels. The LNT hypothesis states that risk per unit dose is constant without a threshold; i.e. that risk is additive and can only increase. The results in cells and *in vivo* show that risk decreases, rather than increases with increasing dose. This reduction in risk below the spontaneous risk level is also not linear with dose. The decrease appears to reach a maximum with the first track of radiation through the cells, i.e. at the lowest dose physically possible in a cell, and stays at that level until the dose reaches about 100 mGy where risk then rises above the spontaneous level. These results indicate that at low dose rate, the assumption of linearity may be valid only at doses above about 100 mGy (with some variation in different tissue types), and below this

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level radiation-induced protective effects dominate risk. The results in human and other mammalian cells, and in whole animals, described here parallel earlier observations in lower organisms, indicating that these adaptive responses to low doses are not unique to mammals but are part of an evolutionarily conserved response. These protective responses appear to dominate even in individuals that are radiation sensitive and cancer prone for genetic reasons.

Since radiation exposures in the nuclear industry are overwhelmingly in this low dose and low dose rate region, these results should be considered for their implications for radiation protection and industry practices. In particular, the use of the LNT hypothesis for risk estimation at low doses should be reviewed.

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