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LOW DOSES OF RADIATION ARE PROTECTIVE *IN VITRO* AND *IN VIVO*: EVOLUTIONARY ORIGINS

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□ Research reports using cells from bacteria, yeast, alga, nematodes, fish, plants, insects, amphibians, birds and mammals, including wild deer, rodents or humans show non-linear radio-adaptive processes in response to low doses of low LET radiation. Low doses increased cellular DNA double-strand break repair capacity, reduced the risk of cell death, reduced radiation or chemically-induced chromosomal aberrations and mutations, and reduced spontaneous or radiation-induced malignant transformation *in vitro*. In animals, a single low, whole body dose of low LET radiation, increased cancer latency and restored a portion of the life that would have been lost due to either spontaneous or radiation-induced cancer in the absence of the low dose. In genetically normal fetal mice, a prior low dose protected against radiation-induced birth defects. In genetically normal adult-male mice, a low dose prior to a high dose protected the offspring of the mice from heritable mutations produced by the large dose. The results show that low doses of low-LET radiation induce protective effects and that these induced responses have been tightly conserved throughout evolution, suggesting that they are basic responses critical to life. The results also argue strongly that the assumption of a linear increase in risk with increasing dose in humans is unlikely to be correct, and that low doses actually reduce risk.

Keywords: adaptive response; induced radioresistance; low doses, evolutionary conservation; reduced risk; radioprotection

INTRODUCTION

In toxicology, including exposure to chemicals or ionizing radiation, the general regulatory approach in setting limits for human exposure has been the assumption of a linear increase in risk with increasing levels of exposure, with or without a threshold. For ionizing radiation, these recommendations are based on a linear-nonthreshold model, and are contained in a report by the International Commission for Radiological Protection (ICRP 1990). As recently as 2003, the U.S. National Council on Radiation Protection and Measurements concluded "that the weight of evidence suggests that lesions that are precursors to cancer (i.e., mutations and chromosome aberrations), and certain types of cancer as well, may increase in frequency linearly with the dose in the low-dose domain" and "that no alternative dose-response model is more plausible than the linear-nonthreshold model " (Upton 2003).

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The difficulties of this position are, however, becoming increasingly evident (Bonner 2003, Mitchel and Boreham 2000, Parsons 2000). Recently the general assumption of linearity for most toxic agents has been challenged (Calabrese 2004, Calabrese and Baldwin 2000, 2003a, 2003b, Parsons 2002, 2003) and it has been argued that beneficial effects arise from exposure to low levels of a wide variety of agents, including ionizing radiation, that are generally considered to be detrimental to human health at high levels. This paper considers that hypothesis, variously referred to as an adaptive or hormetic response, for exposure to oxidative stress in the form of ionizing radiation, and examines the question from the point of view of an evolutionary conservation of biological responses to low-dose ionizing radiation exposure.

EXPERIMENTAL STUDIES

Prokaryotes

Exposure of bacteria to stress, including oxidative stress, induces resistance to the lethal effects of radiation exposure. Pre-exposure of *Vibrio cholerae* cells to X-rays or hydrogen peroxide made the cells about 3-fold more resistant to a subsequent challenge by X-rays (Basak 1996). Exposure of *E. coli* to heat stress invoked resistance to oxidative stress (Delaney 1990) and exposure to oxidative stress in the form of ionizing radiation induced resistance to killing by further radiation exposure (Pollard et al. 1981), as did exposure to the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (Morse and Smith 1987). This induced radiation resistance required protein synthesis and resistance developed only if the cells were recA+ and lexA+, indicating that induced S.O.S. repair was involved. In bacteria, this type of repair is a recombinational type of DNA repair that requires another homologous genome, and is necessary to repair damage occurring in both strands of a duplex at the same place, such as a double-strand break or an interstrand crosslink. DNA double strand breaks are responsible for the chromosomal breaks and aberrations characteristic of ionizing radiation exposure. However, other DNA repair systems are also induced, including DNA excision repair (Huang and Claycamp 1993). It has been estimated that the threshold dose for induction of such repair and radioresistance by X-rays in prokaryotes may be as low as 1 mGy (Ewing 1995).

Lower Eukaryotes

The evolutionary progression from prokaryotes to eukaryotes is characterized by the acquisition of a nuclear membrane and the organization of DNA into chromosomes. Adaption to radiation has been examined in the simplest of the eukaryotes, the single celled organisms. One of these, yeast, has been used extensively to examine adaptive responses to stress,

including both heat and ionizing radiation. Exposure of *Saccharomyces cerevisiae* to non-lethal doses of gamma radiation, or to heat stress, has been shown to increase their resistance to killing by a second exposure of either stress, a process requiring protein synthesis (Mitchel and Morrison 1982a). One stress could induce the maximum resistance to the other stress (Mitchel and Morrison 1984a), suggesting that the same processes were involved in the responses to either stress and that the responses are a general response to stress, rather than a unique response to a specific stress. In wild type diploid yeast, this adaptive response to radiation was correlated with an increase in the cell's maximum capacity for homologous recombination (Mitchel and Morrison 1982b). The response was absent in diploid yeast defective for homologous recombination, and in haploid yeast, increased radiation resistance was seen in cells in the G2 phase of the cell cycle, but not in the G1 phase (Mitchel and Morrison 1982a, Dolling et al. 2000), indicating that increased radioresistance required a duplicate copy of the genome. Taken together, this is compelling evidence that adaption to radiation resulted from an increased capacity for homologous recombination, a particular type of relatively error-free DNA repair. If these adapted yeast were subsequently exposed to any of a variety of chemical mutagens, they showed increased resistance to mutagenesis, indicating that the DNA repair capacity, elevated by radiation exposure, also reduced the risk from chemical mutagen exposure (Mitchel and Morrison 1987). In yeast, the increase in homologous recombination type of DNA repair was proportional to the exposure, up to a limit. Above that dose limit, no further resistance developed (Mitchel and Morrison 1982b).

This adaptive response in yeast also showed an "oxygen effect", whereby exposure in the presence of oxygen produced a greater adaptive response than exposure in the absence of oxygen (Mitchel and Morrison 1984b, 1987). Oxygen effects are well known in radiobiology, and are thought to reflect a greater yield of chemically unrepairable DNA damage due to the reaction of free radical sites on DNA with oxygen. In the context of adaption, these results indicated that DNA damage serves as the signal for the induction of elevated capacity for DNA repair. Experiments comparing high-LET radiation (neutrons) to low-LET radiation (gamma-rays) explored the nature of the DNA damage to which the cells were responding. The gamma-rays were shown to be more effective, per unit dose, than neutrons at inducing the adaptive response (Boreham and Mitchel, 1990). Since gamma-rays produce relatively more DNA single strand breaks, and relatively less DNA double-strand breaks, than neutrons, this result suggested that the yeast responded more strongly to single-strand break types of DNA damage. Further experiments suggested that the signal for induction of radioresistance genes was not likely DNA damage *per se*, but more likely local distortions in the DNA

topology (Boreham et al. 1990), a suggestion that also accommodated the observed induction of the response by heat stress.

The involvement of stress protein induction in the induction of radioresistance in yeast was examined by Boreham and Mitchel (1994). They showed that HSP 104, an inducible heat-stress protein, while not essential for induction of radioresistance, was important in regulating the development and magnitude of the response.

A study of the adaptive response to radiation in another single-cell eukaryote, the alga *Chlamydomonas reinhardtii*, showed a strong adaptive response that was proportional to the inducing radiation dose (Chankova and Bryant 2002). This dose-dependent increase in radioresistance was correlated with a dose-dependent increase in DNA double-strand-break repair rate, a finding that parallels the observations in yeast showing that the extent of adaption to radiation was also proportional to dose, and was dependent on induction of homologous recombination type of DNA repair.

The next level of eukaryotic organisation is typified by eukaryotes with limited cellular organization, such as the nematode *Caenorhabditis elegans*. Pre-exposure of wild-type *C. elegans* to oxidative stress conferred a protective effect against the lethal effects of subsequent X-irradiation (Yanase et al. 1999).

Taken together, these results show that the underlying mechanisms of the adaptive response to an oxidative stress such as radiation has been conserved from prokaryotes to lower eukaryotes, and that this adaptive response has been further preserved when eukaryotes became organized into simple multicellular organisms.

Higher Eukaryotes

Plants

Cortes et al. (1990a) examined plant cells for evidence of an adaptive response to radiation. When *Allium cepa* root-tip cells were exposed to low doses of X-rays or to incorporated tritium and subsequently given a 'challenge' dose of X-rays, the X-ray-induced chromosomal damage was reduced, indicating an adaptive response capable of repairing DNA double-strand breaks exists in plants.

Insects

Adaption to radiation has been shown in insects. Experiments in lepidopteran cells (Koval, 1988) showed that the survival of cells plated immediately following the second of two equivalent doses separated by several hours was greater than the survival of cells plated immediately following a single dose equal to the sum of the split doses. The survival was approximately five times greater than that expected after the second dose, suggesting that the first split dose stimulated a repair system not

present in unirradiated cells. In other experiments, exposure of flour beetles to gamma-rays enhanced longevity (Ducoff 1975) and induced resistance to oxygen toxicity (Lee and Ducoff 1984). The magnitude of the effect was dose-dependent, and irradiation under anoxic conditions reduced the development of oxygen resistance to the same degree that it reduced acute radiation lethality, a dose dependence and radiobiological oxygen effect similar to the observations in the simple eukaryote yeast (Mitchel and Morrison 1982a, 1984b, 1987). In Drosophila, induction of the adaptive response to radiation in mature oocytes was at a maximum after an adapting dose of 0.2 mGy (Schappi-Buchi 1994), suggesting that the cells respond to first track of radiation, in a manner similar to prokaryotes (Ewing 1995).

Fish

Kurihara et al. (1992) examined the response of fish cells to low doses of X-rays. They showed that in cells cultured from two species, either goldfish or mudminnows, low doses of radiation reduced both chromosomal aberrations and the frequency of micronuclei resulting from a subsequent exposure to a high dose of radiation, indicating that the low dose had increased the ability of the fish cells to repair DNA double strand breaks.

Mammalian cells

DNA repair and mutagenesis. Exposure of human lymphocytes to beta-radiation by culture in the presence of tritiated thymidine decreased the yield of chromosomal aberrations from a subsequent X-ray exposure (Olivieri and Wolff 1984). A similar observation was made using gamma-rays and lymphoblastoid cells (Rigaud 1993). The protection was not due to a diffusible factor and was consistent with a chromosomal repair mechanism (Wiencke 1986). Doses as low as 1 cGy of X-rays protected against DNA double strand breaks induced by either radiation or chemicals (Wolff 1988), a finding that is consistent with observations in both bacteria and yeast. Other oxidizing agents such as hydrogen peroxide also induced the protective effect in human lymphocytes (Cortes 1990b), as well as in bovine or rabbit lymphocytes (Flores 1996). This adaptive process required protein synthesis (Youngblom 1989), a characteristic also conserved from prokaryotes.

Low doses and low dose-rate exposures have also been shown to increase the ability of human skin fibroblasts to repair radiation-generated breaks in chromosomes, as shown by an increased rate of repair (Azzam et al. 1994a). An increase in the rate of an enzymatic process implies either elevated enzyme levels or increased access to the substrate. The enhanced repair occurred after 1 mGy, which represents, on average, a single track per cell (Broome et al. 2002). Higher doses, representing multiple tracks/cell, produced the same result as one track/cell when

those tracks from the higher doses were spaced out in time. This ability of human cells to respond to the first track of radiation also appears to have been conserved from prokaryotes (Ewing 1995) and insect cells (Schappi-Buchi 1994).

In mammals, the induced protection is not unique to human cells. Cultured Chinese hamster V79 cells exposed to chronic beta-rays from incorporated tritiated thymidine also showed reduced sister-chromatid exchange and micronucleus formation when subsequently exposed to a high dose of gamma-rays (Ikushima 1987). When exposed to a low dose of gamma-rays 4h prior to a challenge dose, the rate of DNA damage repair in the adapted cells was higher than that in non-adapted cells, and the residual damage was less in adapted cells than in non-adapted cells. In these cells, there was no indication of any difference in the initial yields of DNA double-strand breaks induced by challenging doses from non-adapted cells and from adapted cells (Ikushima et al. 1996), indicating that the predominant feature of the induced resistance was enhanced DNA repair capacity, not a reduction in the initial damage by, for instance, elevated radical scavenging.

The enhanced repair capacity in adapted cells also protected against mutation in human-hamster hybrid cells, where a low dose followed by a later challenge dose significantly reduced the yield of mutants compared to the challenge dose alone. As observed for enhanced resistance to killing and enhanced DNA repair, incubation with a protein synthesis inhibitor largely negated the decrease in mutant yield (Ueno et al. 1996). The reductions in chromosomal aberrations and mutation yield observed in the various mammalian cells used for these low dose studies implies that the net error rate for the overall DNA repair must have been reduced in adapted cells, further implying that the induced repair process is relatively error-free. The reduction in mutations in radiation adapted mammalian cells was also seen in a single cell eukaryote, indicating evolutionary conservation of this feature of the stress response.

Exposure to low doses has also been shown to induce an adaptive response in cells taken from wild white-tailed deer. Exposure to doses as low as 1 mGy increased the ability of skin fibroblasts to repair chromosomal breaks from a second high dose (Ulsh et al. 2004), a response that replicates that observed in human skin fibroblasts exposed to the same low dose (Broome et al. 2002), and that was also seen in prokaryotes (Ewing 1995).

When lower eukaryotic cells were exposed to a heat shock, which induces heat-shock proteins, the cells became radioresistant (Mitchel and Morrison 1982a) and the extent of the radioresistance was regulated by a heat shock protein (Boreham and Mitchel 1994). This association of heat-shock protein induction with induction of radioresistance has apparently also been conserved in mammalian cells. When RIF cells, which did not

show an adaptive response to radiation, were transfected with inducible HSP70 or HSP25, the adaptive response was restored and both radioresistance, measured by a clonogenic assay, and a reduction of apoptosis were detected in these cells (Kang et al. 2002, Lee et al. 2002). In human lymphocytes, either a mild heat stress or a low dose of ionizing radiation induced an adaptive response that protected against cytogenetic damage from a subsequent high radiation exposure (Cai and Jiang 1995). When the two adaptive treatments were combined, no additive effects on the magnitude of the adaptation induced were observed, suggesting that low-dose radiation and hyperthermia may share one mechanism of induction of adaptation to cytogenetic damage. This same observation has been reported in the single cell eukaryote *S. cerevisiae* (Mitchel and Morrison 1982a, 1984a), again indicating that adaption to radiation is part of a general stress response conserved from lower to higher eukaryotes.

Although the studies noted above clearly show that low doses of radiation or other oxidizing stress induce DNA repair processes that increase the ability of the cell to repair broken chromosomes, and reduce mutation and chromosomal aberrations, these endpoints are not the direct measures of risk, such as cancer, usually considered when assessing radiation risk. However, the impact of these evolutionarily conserved, inducible protective effects on the risk of malignant transformation has been tested in both rodent and human cells.

Malignant transformation. The essential features of the oxidative stress-inducible DNA-repair processes appear to have been tightly conserved during the evolution of prokaryotes to mammalian cells, including human cells, a strong argument that this process is highly important to life. However, this does not necessarily indicate a reduction in the risks normally associated with and measured after a radiation exposure. The most important of those risks is generally considered to be cancer. One characteristic of cancer cells is genomic instability. Suzuki et al. (1998) showed that low doses reduced the level of genomic instability induced in human cells by a high dose. Azzam et al. (1994b) measured both the ability of cells to repair chromosome breaks (DNA double-strand breaks) and the frequency of malignant transformation in rodent cells exposed to a high dose and dose rate of gamma radiation, with and without a prior low-dose and low-dose-rate exposure. The prior low dose, given 24h before the high dose, reduced both the frequency of unrepaired chromosomes and the frequency of malignant transformation induced by the high dose, indicating that the enhanced DNA repair was relatively error-free, and that the low-dose exposure was actually reducing cancer risk. Evidence presented by Broome et al. (1999) indicated that the application of the induced repair in human cells was biased toward some chromosomes and away from others suggesting that the decrease in risk may depend on or reflect this bias.

The same group extended those experiments by testing the effects of low gamma-radiation doses alone (without a second high dose) on spontaneous malignant transformation in rodent cells (Azzam et al. 1996). Doses between 1 and 100 mGy, given at low dose rate, reduced the risk of spontaneous malignant transformation by about 3-4 fold, and all doses were equally effective. This same concept was tested in human cells by Redpath et al. (1998, 2001) who reached a similar conclusion. These results demonstrate the tight evolutionary conservation, from prokaryotes (Ewing 1995) to complex invertebrates (Schappi-Buchi 1994) to human cells (Broome et al. 1996), of the ability of very low doses of radiation to induce DNA double strand break repair in cells, and the importance of that conserved response for the reduction of risk in mammalian cells, including human cells.

The lowest dose tested by Azzam et al. (1996) and Redpath et al. (2001), 1 mGy of gamma-radiation, represents an average of 1 ionization track per mammalian cell nucleus. That data shows that mammalian cells respond to the first track of radiation, and that the response reduces risk. Since radiation tracks are random, at 1 mGy most cells receive one track but some receive none or more than one. However, all respond to the same extent as they did when they certainly received one or more tracks at the higher (10-100 mGy) doses. This maximum risk-reduction effect at 1 mGy parallels the results on induced DNA double strand break repair (Broome et al. 1996, Ulsh 2004). 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.

Control of adaptive response. These observations in mammalian cells allow an interesting comparison with the induced DNA repair process in lower eukaryotes. Although the data noted above strongly indicate that the fundamental process has been tightly conserved, the results also indicate that the strategy for control of the process has evolutionarily diverged. Although all prokaryotic and eukaryotic cells appear to respond to the first track of radiation, in lower eukaryotes the extent of induction of the DNA repair process by radiation, and therefore the net effect of the process, is clearly dose-dependent. The extent of induction increased with dose (to a maximum induction) (Chankova and Bryant 2002, Le and Ducoff 1984, Mitchel and Morrison 1984b, 1987) and therefore the degree of protection against, for example, cell killing by a second dose or mutation by a chemical mutagen, was also dose dependent. In contrast, the response of mammalian cells to the first track of radiation appears to be amplified, via signalling to bystander cells, to produce a maximum response in some volume of tissue (Broome et al. 2002, Ulsh et al. 2004). This maximum response to the first track, rather than a graded response with increasing dose, could reflect the necessity

to provide maximum protection in the more radiation-sensitive mammalian cells.

The cellular data strongly suggest that the biological processes that respond to radiation are inducible and protective, and that these processes have been tightly conserved throughout evolution. The data also suggest that these processes are likely to impact on risk in whole organisms, and in ways that would preclude a linear increase in risk with increasing dose.

Studies in animals and humans

Experiments in cells have provided compelling evidence that radiation-inducible DNA repair is a basic response to stress that has been closely conserved during evolution, and have additionally provided information about the molecular and cellular events that constitute this important response to low doses. Ultimately however, tests on the implications of these responses on measures of risk must be conducted in whole mammals.

Chromosomal changes

In vivo exposure of higher eukaryotes, including humans, can induce protective effects in cells that are similar to those seen using cells in culture. Exposure of rabbits to low doses prior to a challenge dose *in vivo* reduced the frequency of chromatid aberrations seen in bone marrow (Cai and Liu 1990). Liver cells taken from frogs living in radioactively contaminated ponds (1 mGy/y) were resistant to chromosomal breakage from high doses (preliminary data of Stuart and Morrison, described in Mitchel 2004). This result in frogs was also observed in lymphocytes, taken from humans living in a high background area (260 mSv/y), that showed a reduced level of chromosomal aberrations after a high challenge dose *in vitro*, compared to people living in a nearby area without the high background (Ghiassi-nejad et al. 2002). In similar studies of lymphocytes taken from occupationally exposed nuclear workers (Barquinero et al 1995, Thierens et al. 2002) or medical radiation workers (Gourabi and Mozdarani 1998), the occupational exposures were shown to increase the capacity of lymphocytes to repair broken chromosomes (DNA double strand breaks) resulting from an *in vitro* high dose exposure. These data show that environmental and occupational exposures induce the same protective response in mammalian and amphibian cells that has been seen in prokaryotic and eukaryotic cells grown in culture, and support the idea that this response is relevant to real-life situations in mammals and non-mammals.

Cancer Risk

The studies noted above indicate that low doses induce an evolutionarily conserved DNA repair system that reduces the risk of spontaneous and radiation induced chromosomal aberrations, mutations and malig-

nant transformation. Several studies show that low doses also reduce the risk of cancer in animals.

Exposure of mice with a high spontaneous risk of lymphoma to 15 or 5 cGy two or three times per week respectively, for about 29 weeks significantly reduced the lymphoma incidence and increased the lifespan of the mice (Ishii 1996). A single exposure of mice to 100 mGy at low dose rate increased the latency of myeloid leukemia in mice subsequently exposed to a high dose (Mitchel et al. 1999). These data suggest that a major influence of a single low dose may be a reduction in the rate of progression of genomic instability (the process which controls tumor latency) and multiple exposures may extend tumor latency beyond normal lifespan. Low adapting doses have been shown to reduce genomic instability induced by high doses in human cells in culture (Suzuki et al. 1998).

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

Mice that are heterozygous for the *Trp53* gene (*Trp53* +/-) are compromised in their ability to repair DNA damage and in their ability to initiate cell death in improperly repaired cells, two of the processes most important for controlling cancer risk.

Consequently, such mice are cancer-prone (Kemp 1994). A dose of 10 mGy, given at low dose rate the day before a 4 Gy exposure delayed the onset of lymphomas in *Trp53* +/- mice, but did not change their frequency (Mitchel et al. 2004) paralleling the effect for myeloid leukemia seen in genetically normal (T*rp53* +/+) mice (Mitchel et al. 1999). Increasing the low adapting dose to 100 mGy caused this protective effect to disappear in the *Trp53* +/- mice. While not increasing harm, 100 mGy apparently represented an upper threshold for doses that are protective against radiation-induced lymphomas in the cancer-prone mice. In the normal mice, protection was still seen at 100 mGy indicating a higher upper threshold for protective effects in the *Trp53* +/+ mice.

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 influence of induced protective processes on the dominant biological outcome of such exposures. However, to estimate risk for radiation protection purposes it is more important to understand the influence of low doses alone on spontaneous cancer risk. In cancer prone *Trp53* heterozygous mice exposed to low doses, the tumor types and frequencies did not change, indicating that the tumors appearing after irradiation were the same spontaneous tumors seen in the absence of radiation. However, a single exposure of either 10 or 100 mGy, given at low

dose rate to young mice, increased lymphoma latency and restored a portion of the lifespan lost due to the disease in the unexposed, cancer-prone mice (Mitchel et al 2003). In the same mice, the single exposure to 10 mGy delayed the appearance of spontaneous spinal osteosarcomas, but increasing the dose to 100 mGy resulted in a general acceleration of their appearance. Since, in the same animals, the upper dose threshold for protection against lymphomas exceeded 100 mGy, the dose threshold, where protective effects give way to detrimental effects, must be tissue-type specific. The data show, therefore, that protective effects against cancer show thresholds, a result also observed for malignant transformation in human cells (Redpath 2001). The observations indicate that the main effect of a single low dose exposure in animals was to increase tumor latency, suggesting that the rate of progression of genomic instability is slowed after an adapting dose. This observation is consistent with an evolutionarily conserved induction of the capacity for error-free DNA double-strand-break repair, increasing the probability of correct chromosomal rejoining in genomically unstable cells.

Teratogenic risk

Fetal cells are thought to be especially susceptible to radiation exposure, and the teratogenic effects of radiation are another measure of radiation risk in humans. Temple and Schleifer (1995) exposed chicken embryos *in vivo* to low doses 24h prior to a high dose. They report enhanced regeneration of DNA structure in brain and liver in the embryos exposed to the prior low dose, indicating that adaption to radiation exists in embryonic birds.

Wang et al. (1999) showed a correlation between radiation-induced apoptosis and teratogenesis in mice. That group exposed mouse fetuses on embryonic day 11(during the period of organ development) to a priming dose of 0.3 Gy, which significantly suppressed prenatal death and malformation induced by a challenging dose of radiation on embryonic day 12 (Wang et al. 1998). In a similar experiment by Mitchel et al. (2002), the degree of protection afforded by the low dose was shown to be proportional to *Trp53* gene status, with maximum protection in *Trp53* normal fetal mice, reduced but significant protection in *Trp53* heterozygous mice and sensitization in *Trp53* null mice. These observations show that adaption to radiation exists in fetal mammals as well as birds, and that these responses reduce teratogenic risk. The results showing reduced protection against teratogenesis in *Trp53* heterozygous fetal mice, as compared to *Trp53* normal mice, parallel the observations for reduced protection against cancer in *Trp53* heterozygous mice. Since apoptosis in mammalian cells is closely linked to a lack of, or incomplete DNA repair, these results are consistent with protective effects that result from the induction of error-free DNA repair by low doses of radiation in

fetal mice, with the consequences of that induction under the control of *Trp53* gene activity.

Heritable mutation risk

Radiation protection assumptions also consider the risk that radiation will create heritable mutations. Boreham et al. (2004) have examined the risk of heritable mutations from radiation exposure in mice. In their experiments, male mice were exposed to 1 Gy of gamma radiation, with or without an exposure to 100 mGy 24h prior to the 1 Gy. The exposed mice were held for 10 weeks to remove all radiation-exposed mature sperm and then bred to unexposed females. The offspring were examined for mutations received from the exposed male parent germline stem cells. They observed that the 1 Gy exposure approximately doubled the spontaneous rate of mutation in the offspring, but that the prior exposure to 100 mGy reduced the effect of the 1 Gy such that the resulting mutation rate was not different from the spontaneous rate in the absence of any exposure. This observed reduction in heritable mutations is similar to the observations seen throughout simple and lower eukaryotes, and in other mammalian cells, and is consistent with the induction of a DNA repair system by low doses in the sperm stem cells, correctly repairing subsequent radiation damage, and thereby reducing the frequency of heritable mutations in the offspring of the exposed male mice.

CONCLUSIONS

Induction of radioresistance by exposure to oxidative stress, including low doses of radiation, is part of a general cellular stress response that appeared very early in evolution. A central feature of that induced resistance in prokaryotes is the increased ability to correctly repair DNA double-strand breaks, and this capability appears to have been tightly conserved during evolution, appearing in single-cell eukaryotes, simple eukaryotes, insects, plants, amphibians, and mammals, including human cells. This inducible DNA repair capability appears to have remained part of the general cellular stress response. The review presented here is not meant to be exhaustive in describing the evolutionary breadth of this inducible DNA double-strand-break repair, but only to give some specific examples to show the continuity and consistent features of the process throughout evolution. As might be expected from the cellular evidence of reduced mutation and chromosomal aberrations that correlate with the induction of this DNA repair, exposure to low doses of radiation appears to reduce the risk of cancer, teratogenesis and heritable mutations in mammals. It must be noted that as evolution has proceeded, defence mechanisms against oxidative damage, including ionizing radiation, have become more complex. For example, low doses of radiation are known to also induce radical scavengers (Guo et al. 2003) and elevate

immune function (Liu 2003), as well as induce other types of DNA repair (Le et al. 1998), and these may also contribute to the observed reductions in risk. Nonetheless, the evidence showing that the extent of the risk reduction for cancer and teratogenesis is proportional to *Tp53* gene function argues strongly, that for cancer, (extended time for induced repair, controlled by *Tp53* cell cycle delay) and for teratogenesis (the reduction of apoptosis as a result of increased error-free repair), repair induced by low radiation doses is more important than induction of radical scavengers or elevated immune surveillance. The importance of this radiation-inducible DNA repair system as shown by its evolutionary conservation up to and including human cells, and the associated reduction in risk in animals, indicates that current assumptions about increased risk after low-dose radiation exposure are unlikely to be correct.

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