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Tanya K Day
Flinders University and Medical Centre, Bedford Park, South Australia, Australia

Guoxin Zeng
Flinders University and Medical Centre, Bedford Park, South Australia, Australia

Antony M Hooker
Flinders University and Medical Centre, Bedford Park, South Australia, Australia

Madhava Bhat
Adelaide Hospital, and, University of Adelaide, Adelaide, South Australia, Australia

David R Turner
Flinders University and Medical Centre, Bedford Park, South Australia, Australia

See next page for additional authors

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Tanya K. Day, Guoxin Zeng, Antony M. Hooker □ Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia

Madhava Bhat □ Department of Medical Physics, Royal Adelaide Hospital, and School of Chemistry and Physics, University of Adelaide, Adelaide, South Australia

David R. Turner, Pamela J. Sykes □ Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia

□ The pKZ1 mouse chromosomal inversion assay is the only assay that has detected modulation of a mutagenic endpoint after single whole body X-irradiation with doses lower than 1 mGy. A non-linear dose response for chromosomal inversion has been observed in spleen and prostate between 0.001 mGy and 10 mGy, with doses between 0.005-0.01 mGy causing an increase in inversions and doses between 1–10 mGy causing a reduction below spontaneous inversion frequency. An adaptive response is a decreased biological effect induced by a low radiation dose. Adaptive responses contradict the linear-no-threshold model of risk estimation. We demonstrated that very low (0.001 mGy, 0.01 mGy, 1 mGy and 10 mGy) doses of X-radiation induced a chromosomal inversion adaptive response as measured by a reduction in the frequency of subsequent high dose (1000 mGy) induced inversions in prostate. These are the lowest X-radiation doses reported to induce an adaptive response for any endpoint. Adaptive response experiments were also performed where the high dose was administered four hours prior to a low dose of 0.01 mGy or 10 mGy. In both cases an adaptive response was observed. Identification of the modifying factors involved in the adaptive response may provide candidates for radioprotection.

Keywords: low dose X-radiation, pKZ1 inversion assay, adaptive response, non-linear dose response

INTRODUCTION

It is known that high dose X-radiation is a mutagen and a carcinogen. However, at doses below 100 mGy, the harmful effects of X-radiation are less clear. The pKZ1 mouse chromosomal inversion assay is the only assay where a single whole body X-irradiation with doses lower than 1 mGy has produced a statistically significant modulation of a mutagenic endpoint . A non-linear dose response for chromosomal inversion in pKZ1 spleen and prostate has been previously observed, with doses between 0.005-0.01 mGy causing an increase in inversions and doses between 1–10 mGy causing a reduction below sham-treated inversion frequency (Hooker *et al.*,

Address correspondence to Pamela J. Sykes, Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia 5042. Tel: +61-8-82044379; fax: +61-8-82045114; e-mail: pam.sykes@flinders.edu.au

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2004a). At doses of 100 mGy and greater, an increase in inversions was observed. These results suggest that doses of 0.005-0.01 mGy are more mutagenic than doses of 1–10 mGy and that doses of 1–10 mGy might be anti-mutagenic. An adaptive response is a response to an external stress, such as radiation, which results in a lower than expected biological response to the same or a different stress (Wolff, 1996). Adaptive response studies were performed in order to determine whether low X-radiation doses from the different regions of the non-linear dose response could induce protection from the effects of a subsequent high X-radiation dose, or whether the doses would be additive.

MATERIALS AND METHODS

pKZ1 Transgenic Mice

The pKZ1 transgenic construct was developed by Matsuoka *et al.* (1991) and first described as a mutation assay by Sykes et al (1998). The construct has the *E. coli lacZ* gene in an inverse transcriptional orientation relative to a chicken β-actin enhancer-promoter (EP) complex. Inversions in the transgene are facilitated by mouse recombination signal sequences flanking the *lacZ* gene, placing the *lacZ* gene in the correct transcriptional orientation to the EP complex, resulting in expression of the *lacZ* gene product, β-galactosidase (β-gal) which is detected in tissue sections using the chromogenic substrate, X-gal. All experiments were approved by the Flinders University and the Royal Adelaide Hospital / Institute of Medical and Veterinary Science Animal Ethics Committees.

X-Irradiation of mice

A description of the X-ray exposure system and dosimetry has been published previously (Hooker *et al.*, 2004a). Briefly, treated transgenic and non-transgenic pKZ1 mice were restrained in individual compartments in a 6 mm thick Perspex holder and irradiated with a whole body dose of X-rays using a Philips Orthovoltage deep X-ray radiation therapy unit (250 kV, 15 mA) located at the Royal Adelaide Hospital Radiotherapy Facility, Adelaide. The desired dose was achieved through variation in treatment time, lead filters and source to mouse distance. Independent dosimetry was performed for doses of 1 mGy or less using a Victoreen 450P survey meter. The overall uncertainty of dose delivery was conservatively estimated to be ±20%, which is well below the orders of magnitude differences in dose that were studied. pKZ1 mice were treated with a priming dose of 0.001 or 0.01 mGy (dose-rate 0.0014 mGy/min), 1 mGy (dose-rate 0.34 mGy/min), or 10 mGy (dose-rate 13.9 mGy/minute) followed 4 hours later by a 1000 mGy (dose-rate 180 mGy/minute) challenge dose. Sham-treated transgenic and sham-treated non-transgenic pKZ1 mice were used as controls.

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Histochemical Analysis of Inversions in pKZ1 prostate

The method for histochemical detection of inversions in pKZ1 prostate has been described previously (Hooker *et al.*, 2004b). Mice were sacrificed by CO_2 asphyxiation 3 days after treatment and prostate tissue was removed, embedded in OCT cryoprotectant (Tissue-Tek) and snap frozen. Frozen prostate sections were fixed in glutaraldehyde on slides, stained for 4 hours with X-gal and counterstained with neutral red. Slides were dehydrated with ethanol and xylene, and coverslips were mounted with DPX mounting medium (BDH Laboratories, UK). Sections from a pKZ1 transgenic mouse brain, which stain brightly for *E. coli* β-gal activity, were included as a positive staining control, and prostate tissue sections from non-transgenic mice were included as negative staining controls. Tissues were screened at 500× magnification using an Orthoplan microscope (Leitz, Germany). The number of luminal epithelial cells and the number of inversions were scored for 50 prostatic glandular cross-sections from each mouse. The inversion frequency was calculated by dividing the number of luminal epithelial cells with staining indicative of an inversion by the total number of luminal epithelial cells in the same 50 prostatic glandular cross-sections. Slides were coded by another individual and screened blind to eliminate observer bias. Occasionally non-specific staining was scored in non-transgenic prostate tissue. In order to account for non-specific staining, the inversion frequency for each transgenic treatment group was corrected by subtracting the mean non-specific staining frequency observed in non-transgenic control mice. Comparisons between the corrected inversion frequency in the sham-treated group and each radiation treatment group, and comparisons between the corrected inversion frequency in each priming plus challenge group and the 1000 mGy challenge alone group were performed using a 2-tailed Mann Whitney U test.

RESULTS

An adaptive response to X-radiation in mouse prostate was observed in all priming plus challenge groups. All priming doses caused a similar magnitude of reduction in inversions relative to the 1000 mGy group (Table 1A). The adaptive responses for all priming doses completely protected against the inversions that would have been induced by a single 1000 mGy dose, as well as against a proportion of spontaneous background inversions (Day *et al*, 2006). We have also performed adaptive response experiments where mice were exposed to a high 1000 mGy dose and then 4 hours later they were exposed to a low dose of 0.001 mGy or 10 mGy. In both cases an adaptive response for chromosomal inversions was induced (Table 1B) (Day *et al*, 2007).

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TABLE 1A. Adaptive response for chromosomal inversions in pKZ1 prostate induced by low priming followed by high challenge whole body X-irradiation.

Radiation treatment group (mGy)	Corrected Inversion Frequency (T-NT) ± SE ($\times 10^{-3}$)	Percentage change of corrected inversion frequency in radiation treatment group compared to:	
		0 mGy	1000 mGy
0	2.85 ± 0.26		
1000	5.72 ± 1.08	200 % ↑ *	
10	1.66 ± 0.17	42% ↓ *	
1	1.70 ± 0.20	40% ↓ *	
0.01	3.76 ± 0.28	130% ↑ *	
0.001	2.31 ± 0.27	19% ↓	
10+1000	0.98 ± 0.48	66% ↓ *	165% ↓ #
1+1000	1.88 ± 0.47	34% ↓	134% ↓ #
0.01+1000	1.70 ± 0.57	40% ↓ *	144% ↓ #
0.001+1000	0.93 ± 0.41	67% ↓ *	167% ↓ #

5 ≥ n ≥ 15 transgenic mice; 2 ≥ n ≥ 5 non-transgenic mice.

T: transgenic; NT: non-transgenic.

*: statistically significant (p<0.05) compared to the sham-treated group; #: statistically significant (p<0.05) compared to the 1000 mGy group (2-tailed Mann-Whitney U test). Percentage change was calculated as the difference between (T-NT) after each treatment. Single doses were compared to the sham-treated group, and adaptive groups were compared to both sham-treated and challenge (1000 mGy) groups.

↓: reduction in corrected inversion frequency; ↑: induction in corrected inversion frequency.

TABLE 1B. Adaptive response for chromosomal inversions in pKZ1 prostate induced by high priming followed by low challenge whole body X-irradiation.

Radiation treatment group (mGy)	Corrected Inversion Frequency (T-NT) ± SE ($\times 10^{-3}$)	Percentage change of corrected inversion frequency in radiation treatment group compared to:	
		0 mGy	1000 mGy
0	2.87 ± 0.50		
1000	3.16 ± 0.49	110% ↑	
1	1.29 ± 0.45	55% ↓ *	
0.01	3.55 ± 0.39	120% ↑	
1000+1	1.38 ± 0.20	52% ↓ *	56% ↓ #
1000+0.01	1.35 ± 0.50	53% ↓ *	57% ↓ #

4 ≥ n ≥ 6 transgenic mice; 3 ≥ n ≥ 5 non-transgenic mice.

T: transgenic; NT: non-transgenic.

*: statistically significant (p<0.05) compared to the sham-treated group; #: statistically significant (p<0.05) compared to the 1000 mGy group (2-tailed Mann-Whitney U test). Percentage change was calculated as the difference between (T-NT) after each treatment. Single doses were compared to the sham-treated group, and adaptive groups were compared to both sham-treated and challenge (1000 mGy) groups.

↓: reduction in corrected inversion frequency; ↑: induction in corrected inversion frequency.

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The maximum number of inversions per glandular cross-section varied after treatment with single radiation doses ranging between 0.001 and 1000 mGy. There were 0–7 inversions per transgenic prostatic glandular cross-section, and 0–3 staining events per glandular cross-section in sham-treated transgenic or non-transgenic mice. The distribution of inversions in individual prostatic glandular cross-sections was similar between all priming plus challenge groups. The maximum number of staining events per glandular cross-section was always higher in the transgenic than the non-transgenic group for each treatment group. If the inversion frequency was increased when mice received a single radiation dose, then the maximum number of inversions per glandular cross-section was correspondingly increased, and when the inversion frequency was decreased, the maximum number of inversions per glandular cross-section was also reduced. In contrast, in the priming plus challenge groups, although there was an increase in the maximum number of inversions per glandular cross-section, there was not a corresponding increase in the inversion frequency. The difference between the distribution of inversions in prostatic glandular cross-sections between two-dose and single dose treatment groups allowed the generation of a hypothetical model of radiation damage responses. This model of radiation damage response suggested that the chromosomal inversion adaptive response is largely the result of a low dose response, because high dose induced inversions remained in the presence of an adaptive response.

In the present study the induction of a chromosomal inversion adaptive response by low dose radiation either before or after high dose exposure suggests that radioprotective factors do not have to be present at the time of high dose exposure to result in protection from chromosomal inversions.

DISCUSSION

Here we show that adaptive responses for inversions in pKZ1 prostate can be induced by single doses as low as 0.001 mGy, doses which are relevant to population exposures. A dose of 0.001 mGy is three orders of magnitude lower than has previously been shown to cause an adaptive response for any end-point. No correlation was observed between the magnitude of the priming dose and the magnitude of the adaptive response in pKZ1 prostate, as measured by chromosomal inversion frequency. This is in agreement with the only other report of the effect of a range of priming doses between 1–500 mGy on the magnitude of the adaptive response for micronucleus formation, measured using a high challenge dose of 4000 mGy (Broome *et al.*, 2002), and suggests that the adaptive response is an all-or-none process, rather than a dose-dependent one. Non-specific staining was observed in non-transgenic prostate after

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high dose irradiation, despite performing histochemical staining at a pH that was selective for the activity of *E. coli* transgene-specific β-gal rather than mammalian lysosomal β-gal. It seems likely that non-specific staining in pKZ1 prostate after high dose irradiation is related to a cell death process, as radiation-induced β-gal staining in non-transgenic mice has been linked to the clearance of apoptotic cells (Lorimore *et al.*, 2001). β-gal is expressed *in vitro* in senescent cells (Dimri *et al.*, 1995), and therefore induced senescence is also a potential reason for non-specific β-gal expression. Although the mechanisms underlying non-specific β-gal staining in prostate are unknown, there appeared to be no adaptive response for non-specific staining in the priming plus challenge groups suggesting that the adaptive response is protective against DNA inversions but not cell death.

The low followed by high dose and high followed by low dose adaptive response results presented here indicate that the response to high dose radiation is not just a greater magnitude of the response induced by low dose radiation, suggesting that genes involved in the low dose inversion response and high dose inversion response are different. Previous microarray studies have demonstrated that different genes are involved in low and high dose radiation responses (Ding *et al*, 2005)

The distribution of inversions within prostatic glandular cross-sections suggests that the adaptive response is due largely to a reduction in the low dose induced inversion response, rather than the high dose induced inversion response, as high dose induced inversions identified by glandular sections containing more than 4 inversion events were disproportionately maintained in priming plus challenge groups even though the overall frequency of inversions was reduced to below the sham-treated inversion frequency.

The mechanisms underlying the adaptive response are currently unknown, but are presumably the same for priming dose alone and priming plus challenge studies. It is known that DNA double strand break repair is faster and more complete after priming plus challenge irradiation, which suggests a role for DNA repair in the adaptive response (Ikushima *et al.*, 1996). The NEOTRANS$_3$ model was developed to predict and explain non-linear responses for radiation-induced damage (Scott, 2005; Scott *et al.*, 2007). NEOTRANS$_3$ was developed using pKZ1 spleen data (Hooker *et al.*, 2004a) and *in vitro* neoplastic transformation data (Azzam *et al.*, 1994; Azzam *et al.*, 1996; Redpath and Antoniono, 1998; Redpath *et al.*, 2001). Using NEOTRANS$_3$, adaptive responses for chromosomal inversions in pKZ1 prostate were predicted to be induced by the priming doses used here. The underlying basis for the NEOTRANS$_3$ model is low fidelity, error-prone DNA repair in co-operation with apoptosis in the ultra-low dose region below 0.01 mGy, and p53-dependent, high fidelity DNA repair and apoptosis in cooperation with p53-inde-

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pendent protective apoptosis mediated (PAM) processes in the low dose region around 1–10 mGy. The 1000 mGy challenge dose is hypothesised to further activate repair but not inhibit the low dose processes, which act on high dose radiation-induced damage and result in an inversion adaptive response. The adaptive responses observed here suggest that the net effect of the induced radioprotective mechanisms on subsequent high dose induced chromosomal inversions in pKZ1 prostate are similar in the 0.001–10 mGy priming dose range, although different priming doses may activate different types of repair. Studies on apoptosis and p53 expression at the low priming doses used here are required to substantiate the NEO-TRANS₃ model, particularly for two-dose adaptive response studies. Further investigation into the timing of adaptive responses at the 0.001–10 mGy low radiation doses is also required.

The results presented here suggest that radioprotective factors are induced at biologically relevant doses. This finding is likely to have implications for radiation risk assessment and radiation safety and provides further evidence against the validity of the LNT model of risk assessment.

REFERENCES

Azzam EI, Raaphorst GP and Mitchel RE. 1994. Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells. Radiat Res 138: S28-S31

Azzam EI, de Toledo SM, Raaphorst GP and Mitchel RE. 1996. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat Res 146: 369-73

Broome EJ, Brown DL and Mitchel RE. 2002. Dose responses for adaption to low doses of (60)Co gamma rays and (3)H beta particles in normal human fibroblasts. Radiat Res 158: 181-6

Day TK, Zeng G, Hooker AM, Bhat M, Scott BR, Turner DR and Sykes PJ. 2006. Extremely low priming doses of X radiation induce an adaptive response for chromosomal inversions in the pKZ1 mouse prostate. Radiat Res 166: 757-766

Day TK, Zeng G, Hooker AM, Bhat M, Scott BR, Turner DR and Sykes PJ. 2007. Adaptive response for chromosomal inversions in pKZ1 prostate induced by low doses of X radiation delivered after a high dose. Radiat Res 167: 682-692

Ding L, Shingyoji M, Chen F, Hwang J-J, Burma S, Lee C, Cheng J-F and Chen DJ. 2005. Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: a comparative study of low and high doses. Radiat Res 164: 17-26

Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M and Campisi J. 1995. A Biomarker that Identifies Senescent Human Cells in Culture and in Aging Skin *in vivo*. Proc Natl Acad Sci U S A, 92: 9363-9367

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Hooker AM, Bhat M, Day TK, Lane JM, Swinburne SJ, Morley AA and Sykes PJ. 2004a. The linear no-threshold model does not hold for low-dose ionizing radiation. Radiat Res 162: 447-52

Hooker AM, Morley AA, Tilley WD and Sykes PJ. 2004b. Cancer-associated genes can affect somatic intrachromosomal recombination early in carcinogenesis. Mutat Res 550: 1-10

Ikushima T, Aritomi H and Morisita J. 1996. Radioadaptive response: efficient repair of radiation-induced DNA damage in adapted cells. Mutat Res 358: 193-8

Lorimore SA, Coates PJ, Scobie GE, Milne G and Wright EG. 2001. Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20: 7085-95

Matsuoka M, Nagawa F, Okazaki K, Kingsbury L, Yoshida K, Muller U, Larue DT, Winer JA and Sakano H.(1991). Detection of somatic DNA recombination in the transgenic mouse brain. Science 254: 81-86

Redpath JL and Antoniono RJ. 1998. Induction of an adaptive response against spontaneous neo-plastic transformation *in vitro* by low-dose gamma radiation. Radiat Res 149: 517-20

Redpath JL, Liang D, Taylor TH, Christie C and Elmore E. 2001. The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. Radiat Res 156: 700-7

Scott BR. 2005. Stochastic Thresholds: A Novel Explanation of Nonlinear Dose-Response Relationships for Stochastic Radiobiological Effects. Dose-Response 3: 547-67.

Scott BR, Haque M and Di Palma J. 2007. Biological Basis for Radiation Hormesis in Mammalian Cellular Communities. Int J Low Radiation, 4:1-16

Sykes PJ, Hooker AM, Harrington CS, Jacobs AK, Kingsbury L and Morley AA. 1998. Induction of somatic intrachromosomal recombination inversion events by cyclophosphamide in a transgenic mouse model. Mutat Res 397: 209-19

Wolff S. 1996. Aspects of the adaptive response to very low doses of radiation and other agents. Mutat Res 358: 135-142