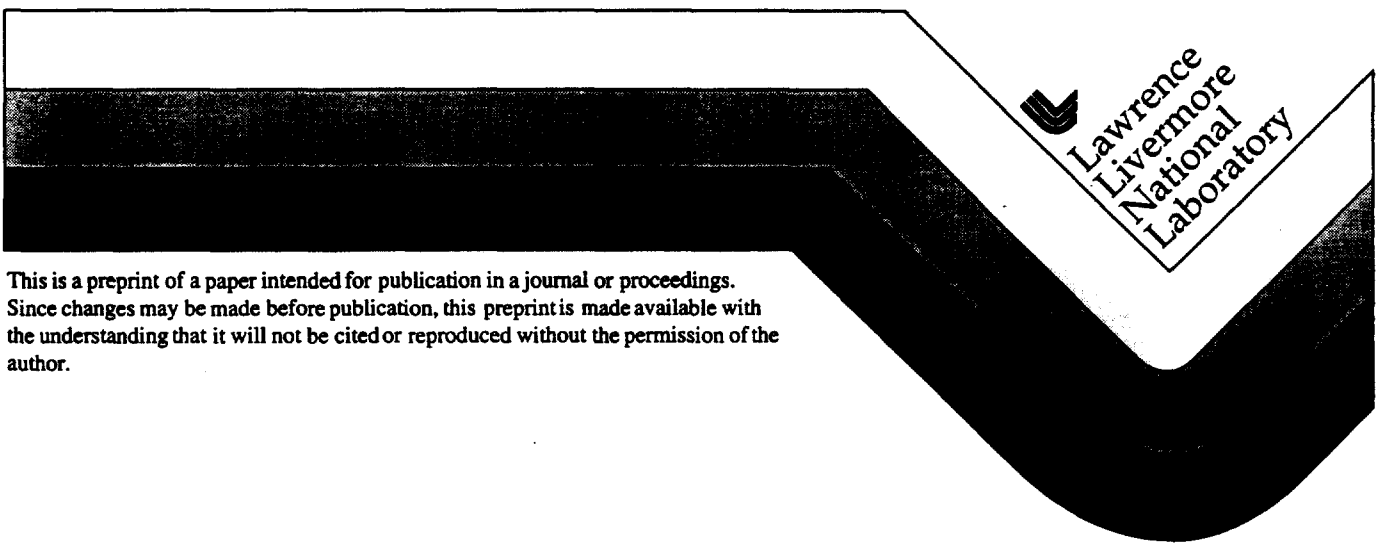
UCRL-JC- 128597
PREPRINT

Chromosome aberrations in lymphocytes of workers occupationally exposed to ionising radiation

Tawn, E. J., Binks, K. , Tucker, J. D., Tarone, R. E.

This paper was prepared for submittal to
Biological Effects and Regulatory Control: IAEA/WHO International Conference on Low Doses of Ionising Radiation, Seville, Spain, November 17 - 21, 1997

September 1997



CHROMOSOME ABERRATIONS IN LYMPHOCYTES OF WORKERS OCCUPATIONALLY EXPOSED TO IONISING RADIATION

E.J. TAWN, K. BINKS, J.D. TUCKER*, R.E. TARONE**

Westlakes Research Institute, Moor Row, Cumbria, CA24 3JZ, UK
* Lawrence Livermore National Laboratory, USA, ** National Cancer Institute, USA

Summary

Cytogenetic studies on workers employed at the Sellafield nuclear installation are reviewed and their relevance discussed in relation to biological dosimetry and risk assessment.

Introduction

Dose response relationships for radiation-induced cancer are derived from epidemiological studies of populations exposed to high doses. However, the classical epidemiological analysis of workforce data lacks the power to establish whether the low levels of exposure encountered occupationally carry any discernible risk to health and current risk estimates for low doses and low dose rates rely on extrapolation from high dose studies and the application of a dose and dose rate effectiveness factor (DDREF) of 2 [1].

Considerable *in vitro* cellular research suggests that the critical target for the deleterious action of radiation is DNA and chromosome aberrations are one of the most thoroughly studied outcomes of the conversion of the initial DNA damage to a mutational endpoint. Most work has concentrated on unstable aberrations, e.g. dicentrics, rings, acentrics, since these can be easily observed, and the dose response kinetics for acute doses over 50 mSv are well established. Indeed, following the finding that for acute exposures the *in vitro* and *in vivo* responses were similar, biological dosimetry based on dicentric frequency in peripheral blood lymphocytes has become a well established technique for assessing the extent of recent acute exposures [2].

Cancer arises as a result of mutation in critical target genes which are involved in the regulation of cell division, cell differentiation, apoptosis and genomic stability. Two types of gene have been identified, proto-oncogenes and tumour suppressor genes. Dominant mutations that convert proto-oncogenes into oncogenes resulting in gain of function are frequently achieved through chromosome translocations. Such chromosome rearrangements are well documented and many exhibit tumour specificity [3]. Mutations in tumour suppressor genes are generally recessive in action and result in loss of function. They can occur by gene mutation or gene deletion, with some deletions encompassing the gene being detectable cytogenetically [3]. Therefore, the types of radiation-induced genetic changes which are consistent with cell viability, e.g. translocations, inversions and small deletions, will be the important endpoints of relevance for malignant transformation. These stable aberrations are more difficult to identify, although classic genetic theory suggests that they should be induced with equal frequency and with the same dose kinetics as their unstable counterparts [4]. Studies of A bomb survivors and radiotherapy patients have shown that cells with stable chromosome aberrations persist for many years following exposure [5,6] and repeated cytogenetic examinations indicate that the frequencies remain unchanged [6], implying constant replenishment by cells derived from aberrant stem cells. The important target cells for carcinogenesis will be the stem cells in the haemopoietic system and other organs but, to date, the relationship between the induction of non-specific chromosome rearrangements and the specific oncogenic mutations necessary for cell transformation is not known.

Cytogenetic Studies on Occupational Radiation Workers

The Sellafield nuclear installation in West Cumbria, UK began activities in 1950 and since 1971 has been operated by British Nuclear Fuels plc. There has been relatively little turnover of the male workforce and there has, therefore, been opportunity for men to be employed as classified radiation workers for long periods of time and thus accumulate relatively high doses of ionising radiation. Cytogenetic studies have been ongoing for many years, initially concentrating on asymmetrical (unstable) aberrations. However cells containing such aberrations will not continue to

undergo repeated successful divisions and therefore more recently G-banding and fluorescence *in situ* hybridisation (FISH) techniques have been employed to identify symmetrical (stable) aberrations. In all the studies chromosome analysis was performed on peripheral blood lymphocytes at their first *in vitro* division. Radiation exposures were measured by external film badge dosimetry [7].

Asymmetrical Aberrations

In 1989 Tawn and Binks [8] reported a study of 71 radiation workers with cumulative radiation exposures >500 mSv and 66 controls with no known previous occupational or medical exposure to clastogenic agents. Cytogenetic analysis using conventional block staining was confined to asymmetrical aberrations (Table I). The mean dicentric frequency for the 71 radiation workers was significantly higher than the control value (p=0.001). However, when the radiation workers were divided into 4 dose groups no significant difference was found between them despite the fact that the men in group 4 had received approximately 2.5 times the mean dose received by group 1. Current smoking data were available. No significant difference was found for dicentric frequency between smokers and non-smokers but when a heavy smoker group, comprising men who admitted to smoking >20 cigarettes per day, was compared to the remainder a significant increase was seen for both controls and the pooled exposed workers. Dicentric frequency rose from $0.34\pm0.24\times10^{-3}$/cell to $2.80\pm2.00\times10^{-3}$/cell in the controls (p=0.05) and from $2.42\pm0.29\times10^{-3}$/cell to $4.71\pm0.82\times10^{-3}$/cell in the radiation workers (p=0.01). There is some evidence to suggest that dicentrics are lost from the peripheral blood with a half-life of approximately 3 years [9]. The dose accumulation pattern for each worker was examined and the annual doses weighted for a 3 year half life and summed to give an equivalent acute dose at time of blood sampling. This was used to establish a dose response for dicentrics. A slope of $1.57\pm0.20\times10^{-2}$/Sv was found when the effect of heavy smoking was considered and $1.44\pm0.20\times10^{-2}$/Sv when heavy smoking was not taken into account.

Table I **Asymmetrical Aberration Frequencies**

Group	Control	1	2	3	4	Total Radiation Workers
No. of individuals	66	15	23	16	17	71
Mean age (y)	40.2	49.5	55.5	56.4	57.0	54.7
Mean total dose (mSv)	-	560	750	950	1230	870
(range)		(510-580)	(630-840)	(900-1000)	(1020-1610)	(510-1610)
Mean radiation work (y)	-	24	29	27	28	27
(range)		(13-33)	(21-33)	(21-31)	(23-30)	(13-33)
Smokers (%)	33	60	61	44	65	58
Dicentrics/cell$\pm$S.E.$\times10^{-3}$	0.61±0.30	2.80±0.61	2.52±0.47	3.25±0.64	3.06±0.60	2.87±0.28
Acentrics/cell$\pm$S.E.$\times10^{-3}$	2.12±0.57	5.47±0.85	3.39±0.54	2.25±0.53	2.71±0.56	3.41±0.31

G-Banding Analysis

The accurate detection of symmetrical aberrations using a G-banding technique is technically demanding and time consuming. Nevertheless interest in establishing a genetic marker for the cumulative effect of chronic low dose radiation led to the study of the 38 radiation workers who comprised the 2 lower dose categories of the previous dicentric study. However, when evaluating frequencies of stable aberrations in relation to radiation exposure the identification of any lifestyle factors involving clastogenic exposure is important since the frequency is likely to reflect a lifetime's exposure to genotoxins. Smoking and age are both significant factors affecting stable aberration frequencies [10,11] and therefore the G-banding data from the 2 radiation worker groups was compared to a subset with the same age profile from a larger study on background frequencies and the data for smokers and non-smokers analysed separately (Table II). The small control smoker group and the inability to quantify lifetime smoking habits made it difficult to deduce anything from the smoking data. The non-smokers demonstrated an increase in dicentrics with increasing dose which did not reach significance but a significant increase with dose was found for translocations (p=0.05)

and for total symmetrical aberrations (p=0.01). Indeed a dose response for translocations of 1.54±0.02x10^{-2}/Sy can be derived from the means of the 3 groups but this simplistic approach hides the variability in the data.

Table II **G-banding Aberration Frequencies**

	Non-smokers			Smokers		
Group	Control	1	2	Control	1	2
No. of individuals	20	6	9	8	9	14
Mean age (y)	51.0	49.5	55.4	52.3	49.2	55.1
Mean total dose (mSv)	-	550	750	-	560	750
(range)		(510-580)	(690-800)		(530-580)	(630-840)
Mean radiation work (y)	-	26.3	30.6	-	23.0	27.9
(range)		(19-33)	(26-33)		(13-31)	(21-32)
Dicentrics/cell±S.E. x 10^{-3}	2.00±1.41	3.33±2.36	4.44±2.21	0	7.78±2.94	2.86±1.43
Translocations/cell±S.E.x10^{-3}	5.00±2.24	13.33±4.71	16.67±4.30	20.00±7.07	13.33±3.85	18.57±3.64
Terminal deletions/cell±x10^{-3}	1.00±1.00	1.67±1.67	4.44±2.21	5.00±3.54	2.22±1.47	2.14±1.24
Asym exchanges/cell±S.E.x10^{-3}	2.00±1.41	5.00±2.89	4.44±2.21	0	10.00±3.33	4.29±1.75
Sym exchanges/cell±S.E.x10^{-3}	8.00±2.83	13.33±4.71	25.56±5.33	25.00±7.91	16.67±4.30	22.86±4.04

Chromosome Painting by FISH

The work described above led to a further collaborative study of a larger group using G-banding and FISH chromosome painting. Tucker *et al* have recently reported the chromosome painting data [12]. Samples from 81 radiation workers, 23 with minimal exposure (<50 mSv) and 58 with exposures ranging from 173 to 1108 mSv, all but 3 being >500 mSv, were examined. Since the painting technique only allows a fraction of all chromosome exchanges to be identified, the results were converted to frequencies for the whole genome. For data analysis the men were evaluated in 5 dose categories (Table III). The mean stable aberration frequencies showed a significant increase with dose category (p=0.032) and with cumulative dose when dose was treated as a continuous variable (p=0.015). When dose and smoking status were considered, a dose response for stable aberrations of 0.79±0.22x10^{-2}/Sv was derived. No significant increase was found for dicentrics. Thus fewer stable aberrations per Sv were observed in these men exposed within permitted limits over several decades than were observed in people receiving acute exposure from the Japanese A-bombs.

Table III **Chromosome Analysis by FISH Chromosome Painting**

Group	1	2	3	4	5
No. of individuals	23	12	15	16	15
No. of smokers	10	6	6	5	5
Mean age±S.E.	54.4±1.6	51.1±1.9	56.3±1.3	53.4±1.4	57.6±0.7
Mean total dose±S.E. (mSv)	9.4±2.1	456.3±41.0	603.9±8.4	708.2±8.3	857.8±27.1
(range)	(1-46)	(173-558)	(565-653)	(660-759)	(768-1108)
Mean radiation work (y)	14.3±2.2	29.9±2.2	32.9±1.1	31.8±1.1	35.5±0.6
Stable aberrations/cell±S.E.x10^{-3}	7.4±1.3	8.6±2.4	10.5±1.9	12.4±2.1	13.9±2.5
Dicentrics/cell±S.E.x10^{-3}	1.6±0.4	1.0±0.6	1.1±0.3	1.7±0.6	1.3±0.5

The most comprehensive A-bomb data comes from a study using conventional staining and DS86 kerma doses, with statistical analysis based on the percentage of cells carrying at least one stable aberration [13]. The analysis applied to the Sellafield data gives virtually equivalent results because few cells had more than one stable aberration. The slopes of the dose response curves are 5.6±0.3x 10^{-2}/Sv and 4.0±0.4x10^{-2}/Sv for Hiroshima and Nagasaki respectively. Dividing these by the observation of 0.79±0.22x10^{-2}/Sv obtained from the Sellafield study results in DDREF estimates of

7.0±2.6 and 5.0±1.5 respectively. These data provide direct human *in vivo* information, on a permanent and stable endpoint, confirming that the effects of radiation delivered at low dose rates are smaller than comparable doses given in a short period of time.

Conclusions

Our studies on occupationally exposed radiation workers confirm that chromosome aberration analysis is a valid method of monitoring radiation exposure. Dicentric frequency provides a good method of dose assessment for acute exposures of recent occurrence whereas translocations, which persist and accumulate, can indicate exposures over many years. For risk assessment, however, the challenge is to define the relationship between the dose response for the non-specific chromosome aberrations reported in these studies and the dose response for the induction and persistence of cancer initiating rearrangements.

Acknowledgements

We thank the Sellafield workforce, their representatives, and the study subjects whose co-operation has made these investigations possible, and the many medical, scientific and technical staff who have contributed to the sample collection and cytogenetic analysis. This work was supported by British Nuclear Fuels plc, the US DOE under Contract No. W-7405-ENG-48 at LLNL and the US NCI Interagency Agreement Nos. NCI/CP 10550-51 and 10561.

References

[1] ICRP. 1990 Recommendations of the International Commission of Radiological Protection. ICRP Publication 60. Ann. ICRP 21 Nos.1-3 (1991).

[2] Biological Dosimetry: Chromosome Aberration Analysis for Dose Assessment. Technical Report 260, IAEA, Vienna (1986).

[3] HEIM, S., MITELMAN, S., Cancer Cytogenetics, 2nd Ed, Wiley-Liss Inc., New York (1995) 536pp.

[4] SAVAGE, J.R.K., "Survival and transmission of symmetrical chromosome aberrations", Proc Sixth Int. Cong. Radiat. Res. (OKADA, S., IMAMURA, M., TERASHIMA, T., YAMAGUCHI, H., Ed.), Jap. Assoc. Radiat. Res., Tokyo (1979) 519-24.

[5] AWA, A.A., "Chromosome damage in atomic bomb survivors and their offspring in Hiroshima and Nagasaki", Radiation-Induced Chromosome Damage in Man (ISHIHARA, T., SASAKI, M.S., Ed.), Alan Liss, New York (1983) 433-54.

[6] BUCKTON, K.E., "Chromosome aberrations in patients treated with X-irradiation for ankylosing spondylitis", Radiation-Induced Chromosome Damage in Man (ISHIHARA, T., SASAKI, M.S., Ed), Alan Liss, New York (1983) 491-511.

[7] KITE, A.V., BRITCHER, A.R., Uncertainties in recorded photon radiation doses at Sellafield, Radiat. Prot. Dosim. **67** (1996) 23-32.

[8] TAWN, E.J., BINKS, K., A cytogenetic study of radiation workers : the influence of dose accumulation patterns and smoking, Radiat. Prot. Dosim. **28** (1989) 173-180.

[9] LLOYD, D.C., PURROTT, R.J., REEDER, E.J., The incidence of unstable chromosome aberrations in peripheral blood lymphocytes from unirradiated and occupationally exposed people, Mutat. Res. **72** (1980) 523-532.

[10] TAWN, E.J., CARTMELL, C.L., The effect of smoking on the frequencies of asymmetrical and symmetrical chromosome exchanges in human lymphocytes, Mutat. Res. **224** (1989) 151-6.

[11] RAMSAY, M.J., MOORE II, D.H., BRINER, J.F., LEE, D.A., OLSEN, L.A., SENFT, F.R., TUCKER, J.D., The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting, Mutat. Res. **338** (1995) 95-106.

[12] TUCKER, J.D., TAWN, E.J., HOLDSWORTH, D., MORRIS, S., LANGLOIS, R., RAMSAY, M.J., KATO, P., BOICE Jr., J.D., TARONE, R.E., JENSEN, R.H., Biological dosimetry of radiation workers at the Sellafield nuclear facility, Radiat. Res. (in press).

[13] AWA, A.A., OHTAKI, K., ITOH, M., HONDA, T., PRESTON, D.L., McCONVEY, M.E., "Chromosome aberration data for A-bomb dosimetry reassessment", New Dosimetry at Hiroshima and Nagasaki and its Implications for Risk Assessment (Proc. No. 9), National Council on Radiation Protection and Measurements, Bethesda (1988) 185-202.

Technical Information Department • **Lawrence Livermore National Laboratory**
University of California • Livermore, California 94551