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Cell survival and radiation induced chromosome aberrations

II. Experimental findings in human lymphocytes analysed in first and second post-irradiation metaphases

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Summary. Human peripheral lymphocytes were irradiated in whole blood with 0.5–4.0 Gy of 220 kVp X-rays and the frequency of chromosome aberrations was determined in 1st or 2nd division metaphases discriminated by fluorescence plus giemsa staining. Using the empirical distributions of aberrations among cells, cell survival and transmission of aberrations were investigated. Considering both daughter cells, we found that 20% of fragments and 55% of dicentrics or ring chromosomes are lost during the 1st cell division; i.e. cell survival rate from 1st to 2nd generation is mainly influenced by anaphase bridging of these two-hit aberrations. Cell survival to 2nd mitosis was calculated considering this situation and compared with the survival derived from the fraction of $M1$ cells without unstable aberrations. The resulting shouldered survival curves showed significantly different slopes, indicating that cell reproductive death is overestimated in the latter approach.

Introduction

After irradiation of human lymphocytes with 5.0 Gy X-rays, Sasaki and Norman [9] have shown that differences exist for the persistence of two-hit aberrations (mainly dicentrics) and of acentrics during cell division. The estimated probability that a dicentric will survive the 1st or 2nd division in vitro (discriminated by [^{3}H]-thymidine autoradiography) was 0.5. About 70% of acentrics were found to be lost at 1st division and the remaining 30% were transmitted as a complete entity to a daughter nucleus, appearing as paired identical fragments in $M2$. Taking into account the influence of cell death and not only the probability of survival of aberrations, Carrano and Heddle [4] showed that in Sasaki and Norman's data the transmission probability of acentrics was underestimated.

The present study reports on the results of the chromosome analysis carried out in FPG-stained (fluorescence plus Giemsa) 1st ($M1$) or 2nd ($M2$)

post-irradiation metaphases of human lymphocytes. Applying the formulae derived in part I of this report [3], transmission and survival parameters of induced aberrations were calculated and compared with published results.

Material and methods

Human peripheral lymphocytes from a healthy male donor were irradiated with 0.5–4.0 Gy of 220 kVp X-rays at 0.5 Gy min^{-1} (4.05 mm Al + 0.5 mm Cu). Irradiation and culture procedures were analogous to our earlier experiments and published elsewhere [11, 12]. Chromosome analysis was carried out either in $M1$ or $M2$ metaphases stained by the FPG technique, [1, 6].

Dicentrics and ring chromosomes, R_c, (9% and 17% of total dicentrics in $M1$ and $M2$, respectively) were pooled as two-hit aberrations. Polycentric chromosomes were evaluated as dicentrics according to the formula: number of centromeres – 1. Acentrics comprise fragments, minutes and acentric rings. They appear as paired identical figures in $M2$. The frequency of $M2$ cells with unpaired configurations of acentrics did not exceed 5% at different doses. Such configurations were considered as preparational artifacts and were not included in the analysis. In $M1$-cells containing several acentrics together with exchange aberrations only one fragment was assigned to each exchange. This seems justified since in a previous irradiation experiment analysed with G-banding we found 90% of major exchanges being complete [2]. The remaining fragments were scored as excess acentrics.

Results

Table 1 gives the distribution of two-hit aberrations with mean $\bar{D}$ and excess acentrics with mean $\bar{X}$ among cells in $M1$. The intercellular distribution of two-hit aberrations with mean $E(D)$, paired acentrics with mean $E(F)$ and paired acentrics in cells without two-hit aberrations with mean F_2, in $M2$ is shown in Table 2. Significant overdispersion is indicated by a dispersion index (variance, σ^2/mean) > 1 and by the magnitude of the test quantity, u, > 1.96 [5, 8, 10].

As demonstrated in the theoretical part of this study [3] the observed distributions are now directly used to predict the transmission and survival parameters. These are: T, the probability of transmission of an acentric as a whole to one daughter nucleus; P' and W', the probabilities of a daughter cell ($M2$) to survive from an $M1$ cell having only one acentric or only one two-hit aberration with its associated acentric, respectively. For the calculation of W' Eq. (7), and of T and P' Eqs. (9)–(11) were used. The result is shown in Table 3.

Estimates of the standard deviations of the parameters were obtained by Monte Carlo simulation. Using the empirical distributions for dicentrics and excess acentrics in $M1$ from Table 1 and the mean of the derived parameters W', T and P' from Table 3, the initial distributions for aberrations in $M2$ were computed according to the model in part I of our study. Then

Table 1. Chromosome analysis in 1st division cells. Intercellular distribution of two-hit aberrations with mean $\bar{D}$ and excess acentrics with mean $\bar{X}$

Case	Dose Gy	Cells scored	Aberrations per cell	Distribution							Dispersion Index σ^2/mean ±SD	u
				0	1	2	3	4	5	6		
$\bar{D}$	0.5	2000	0.034	1933	66	1					1.00±0.03	−0.13
	1.0	1300	0.121	1150	144	5	1				0.98±0.04	−0.46
	2.0	700	0.353	490	177	29	4				0.98±0.05	−0.36
	3.0	600	0.747	282	215	80	19	4			0.97±0.06	−0.46
	4.0	500	1.192	143	193	110	40	9	3	2	0.96±0.06	−0.56
$\bar{X}$	0.5	2000	0.032	1943	52	4	1				1.19±0.03	6.10
	1.0	1300	0.121	1166	112	21	1				1.19±0.04	4.75
	2.0	700	0.343	501	160	37	2				1.02±0.05	0.32
	3.0	600	0.678	297	221	61	20	1			0.95±0.06	−0.91
	4.0	500	1.046	184	174	98	29	10	4	1	1.10±0.06	1.64

Table 2. Chromosome analysis in 2nd division cells. Intercellular distribution of two-hit aberrations with mean $E(D)$, paired acentrics with mean $E(F)$ and paired acentrics in cells without two-hit aberrations with mean F_2

Case	Dose Gy	Cells scored	Aberrations per cell	Distribution				Dispersion Index σ^2/mean $\pm$SD	u
				0	1	2	3		
$E(D)$	0.5	1000	0.016	984	16			0.98 $\pm$ 0.04	−0.35
	1.0	600	0.055	568	31	1		1.01 $\pm$ 0.06	0.13
	2.0	1000	0.178	833	156	11		0.95 $\pm$ 0.04	−1.20
	3.0	300	0.290	220	73	7		0.87 $\pm$ 0.08	−1.55
	4.0	150	0.560	83	53	11	3	0.92 $\pm$ 0.12	−0.67
$E(F)$	0.5	1000	0.018	982	18			0.98 $\pm$ 0.04	−0.39
	1.0	600	0.063	565	32	3		1.10 $\pm$ 0.06	1.69
	2.0	1000	0.190	842	129	26	3	1.18 $\pm$ 0.04	4.02
	3.0	300	0.390	206	72	21	1	1.02 $\pm$ 0.08	0.29
	4.0	150	0.773	72	47	24	7	1.01 $\pm$ 0.12	0.08
F_2	0.5	984	0.012	972	12			0.99 $\pm$ 0.04	−0.26
	1.0	568	0.040	548	17	3		1.22 $\pm$ 0.06	3.83
	2.0	833	0.136	733	86	14		1.11 $\pm$ 0.05	2.26
	3.0	220	0.281	170	38	12		1.11 $\pm$ 0.09	1.16
	4.0	83	0.518	52	20	10	1	1.10 $\pm$ 0.15	0.65

Table 3. Transmission and survival parameters. S.D. estimated by computer simulation

Dose Gy	W'	S.D.	T	S.D.	P'	S.D.	bias
0.5	0.47	0.11	0.38	0.10	1.06	0.50	0.10
1.0	0.45	0.09	0.35	0.06	0.84	0.40	0.08
2.0	0.50	0.05	0.40	0.04	1.34	0.20	0.02
3.0	0.38	0.05	0.42	0.06	1.12	0.30	0.06
4.0	0.45	0.06	0.49	0.05	1.08	0.40	0.11
mean	0.45	0.03	0.41	0.03	1.09	0.17	0.07
[9]	0.53	0.06	0.39	0.08	1.00	0.45	0.09

a sample of a hundred values for each parameter and for each dose was created from the model as described in part I., Sect. 5 by randomly generating hundred frequency distributions for aberrations in $M1$ and $M2$ cells. Derived standard deviations from these samples are shown in Table 3. The high values of the standard deviation for P' confirm the variability of this parameter at the 5 dose points and justify to take the mean.

Repeated simulation procedures revealed that the mean value of P' was higher than the value given to the computer program initially. The excess is listed in Table 3 in the column headed "bias". This may at least partially explain why the computed values for P' in Table 3 were usually >1. Even by subtracting the estimated bias of 0.07 a P' slightly >1 results. Thus there is no evidence to assume $P'<1$ and it can be inferred from our findings

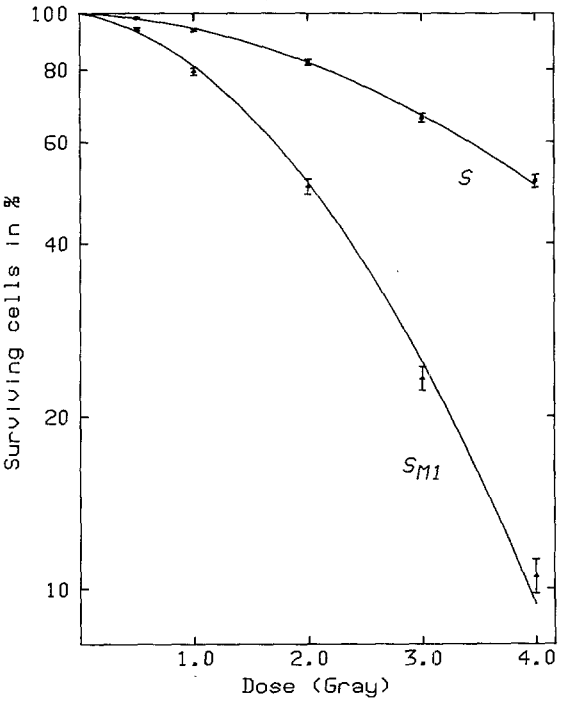

Fig. 1. Percentage of surviving cells $S = S_D S_X$ (■) (S_D, surviving fraction of cells with two-hit aberrations and S_X, with paired excess acentrics, respectively) and of cells without unstable aberrations S_{M1} (▲) against dose for 220 kVp X-rays

Table 4. Expected cell survival at different doses

Dose Gy	S	S_{M1}
0.5	0.981	0.939
1.0	0.935	0.793
2.0	0.823	0.501
3.0	0.661	0.233
4.0	0.512	0.105

that the survival of cells at least from 1st to 2nd generation is not very much affected by the loss of acentrics.

The total survival rate of cells to 2nd mitosis can be calculated from Eq. (4c) $S = S_D S_X$, were S_D and S_X are the independent surviving fractions of cells containing two-hit aberrations or paired excess acentrics, respectively (Eqs. 4a, 4b). Taking $W' = 0.45$ and due to $P' = 1.0$, $S = S_D$. The results are demonstrated in Table 4 and Fig. 1.

For comparison an approach of Lloyd et al. [7] to study the correlation between chromosome aberrations and reproductive cell death is included based on our $M1$ data. The resulting survival, S_{M1}, is given by the fraction of cells without unstable aberrations. It is the product of the relative frequency of cells without dicentrics and R_c and the relative frequency of cells without excess acentrics which can be derived from Table 1. For curve fitting of data from Table 4 the log-linear-quadratic model e^{-Y} with $Y = aD + bD^2$ was used. From a weighted least squares estimation the parameters

Table 5. Survival parameters W and P. S.D. estimated by computer simulation

Dose Gy	W	S.D.	bias	P	S.D.	bias
0.5	0.44	0.26	0.05	1.09	0.75	0.16
1.0	0.54	0.18	0.01	0.72	0.70	0.16
2.0	0.37	0.09	0.00	1.56	0.30	0.05
3.0	0.34	0.13	0.00	1.20	0.50	0.10
4.0	0.42	0.14	0.01	1.17	0.70	0.20
mean	0.42	0.08	0.01	1.15	0.27	0.13
[9]	0.53	0.22	0.03	1.0	0.80	0.23

$a=0.0236\pm0.0043\ \mathrm{Gy}^{-1}$ and $b=0.0369\pm0.0020\ \mathrm{Gy}^{-2}$ were obtained for $\ln S$ and $a=0.0825\pm0.0114\ \mathrm{Gy}^{-1}$ and $b=0.127\pm0.005\ \mathrm{Gy}^{-2}$ for $\ln S_{M1}$. As weight the inverse variance of $\bar{D}+\bar{X}$ was used for S_{M1} and the inverse variance of $(1-W')\,\bar{D}$ for S. This is justified since in the case of Poisson distribution of $\bar{D}$ and $\bar{X}$, Y corresponds to the dose-response relationship of total aberrations $\bar{D}+\bar{X}$ for S_{M1}. For $S=S_D$ it corresponds to the linear-quadratic expansion of $(1-W')\,\bar{D}$ (see Eq. (16) in part I of the present study). It is demonstrated in Fig. 1 that the resulting shouldered survival curves are clearly different. This is also indicated by their different reciprocal initial logarithmic slopes D_1 which were calculated as 42.4 ± 7.7 Gy for S and as 12.1 ± 1.7 Gy for S_{M1}.

Discussion

When Carrano and Heddle [4] recalculated Sasaki and Norman's data [9] the survival and transmission parameters T, P and W could not be determined uniquely since there was no information on F_2, the intercellular distribution of paired acentrics in $M2$ cells without two-hit aberrations. Therefore they assumed a most probable value of $W=0.5$. Using now the observed data they obtained $P\simeq1.0$ and $2T\simeq0.78$ from their formulae which are based on the assumption that radiation induced chromosome aberrations are distributed randomly. The corresponding parameters to W and P are $W'=0.53$ and $P'\simeq1.0$.

By means of our modified formulae each parameter can be calculated using empirical distributions. $T=0.41$ obtained from the present experiment is fairly close to the published result, and $W=0.42$ is slightly smaller. The same holds for $W'=0.45$ (Tables 3 and 5). It is also evident that P and P' can be more precisely determined when higher cell numbers are analysed (Sasaki and Norman's data are based on 200 $M1$ and 157 $M2$ cells).

In general the derived parameters are not linear functions of the aberration yields and frequencies. Moreover, $E(F)$, F_2 and $E(D)$ are stochastically correlated. This means that the expected values of the parameters as functions of the observed aberration frequencies are generally not equal to values which would be derived by inserting the expected but unknown aberration

frequencies into Eqs. (7), (9)–(11) derived in part I of this study. Our simulation experiments revealed a bias for P'. If we look at Eq. (15)

$$P' = \frac{\frac{E(D)}{\bar{X}}}{\frac{E(F)}{F_2} - 1}$$

it may be suspected that P' is the most sensitive parameter since it depends on four variates and their interacting dispersions. If the frequency of two-hit aberrations in $M2$, $E(D)$ is small the denominator of this equation is close to zero and, therefore, P' increases. According to $P' = T + P(1-T)$, P' must vary in the range of $T \leq P' \leq 1.0$. Thus it is acceptable from our data that $P' = P = 1.0$. Applying our simulation experiments for the estimation of the standard deviations (SD) of W, P, W' and P', respectively it is evident from the lower values resulting for SDs of W' and P' that these parameters are more appropriate for an analysis of cell survival.

Considering both daughter cells we determined the probability of fragment transmission to a daughter cell at anaphase as $2T = 0.82$, i.e. only 20% of fragments are lost. The probability that a each daughter cell will receive a two-hit aberration and survive to the next mitosis (fall free of dicentrics or R_c) was calculated as $W' = 0.45$. This means that 55% of these aberrations are lost. Our data clearly show that cell survival rate from 1st to 2nd division is mainly influenced by two-hit aberrations. The loss of fragments seems to be only of secondary importance. Similar conclusions were already made by Carrano and Heddle [4] from Sasaki and Norman's data [9] and are herewith experimentally confirmed.

The existence of different slopes of the two survival curves $S = S_D$ and S_{M1} (Fig. 1) can now be easily explained. If cells containing acentrics retain their capacity of cell proliferation after loss of fragments (whether this holds for later generations cannot be deduced from the present findings) then the influence of cell reproductive death must be over-estimated in the latter approach.

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