PCC-ring induction in human lymphocytes exposed to gamma and neutron irradiation.

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Abstract

Dose-effect curves for dose assessment in Gamma and neutron overexposures to high doses are presented in this paper for the first time in literature. The relationships were obtained by plotting the Premature Chromosome Condensation -rings (PCC-R) frequencies in PCC lymphocytes obtained by chemical induction with Calyculin A *in vitro*, with radiation doses between 5 to 25 Gy. For the elaboration of these curves 9 676 PCC cells in G1 G2 and M stages were analyzed. The results were fitted to a lineal quadratic model in Gamma irradiation. For neutron irradiation the data was fitted to a lineal quadratic model up to 10 Gy, and then a markedly cell cycle arrest and saturation was observed. These curves are of particular interest for victims exposed to doses exceeding 5 Gy where it is always very difficult to estimate a dose using the conventional technique.

1. INTRODUCTION

Premature Chromosome Condensation-ring (PCC-R) in Giemsa stained preparation has become an attractive method for biological dosimetry particularly for high dose exposition. This method proposed by Kanda [1] combines the possibilities of the efficiently premature chromosome condensation induction by okadaic acid or the Calyculin A with the simplicity of the Giemsa stained [2]. This method overcomes three major problems of the conventional biological dosimetry by dicentric analysis at high doses, (i) lymphopenia due to cell death reduces the number of lymphocytes available; (ii) radio-induced cell cycle arrests causes low mitotic index. (iii) dicentrics saturation at high doses. Therefore the mitotic index is low and the number of cells available to have a statistically significant result based on at least 100 cells is very difficult to achieve at high dose. In addition, dose estimation is less precise as the increase rate of dicentrics with dose is less pronounced [1]. PCC is efficiently induced even in cells exposed *in vitro* up to 40 Gy dose radiation [3]. The Tokai-mura accident shows the possibilities to apply this technique in cases exposed up to around 20 Gy of gamma irradiation [1]. However in this accident exposure radiation was a mix of neutron and gamma rays and no PCC ring dose effect curve is available. Up to now just one PCC-R curve is published but without any dose effect relationship to be use to estimate a dose. In the present work this technique is applied *in vitro* for fission neutron

radiation with doses up to 25 Gy. Furthermore, the relevance of this technique is confirmed in the interval of 5-25 Gy for gamma radiation

2. EXPERIMENTAL WORK

2.1 Materials and Methods

Blood sample exposure. Peripheral blood was irradiated with ^{60}Co at 5, 7.5, 10, 20 and 25 Gy (0.5 Gy/min) on ICO-4000 facility at Fontenay-aux-Roses (France). Dosimetry was expressed in terms of tissue Kerma. Another sets of blood samples were exposed to fission neutrons at the SILENE facility (Valduc, France) at the following doses: 5, 7.5, 10, 20 and 25 Gy. The photon and the neutron dose components of the SILENE radiation field were estimated respectively using alumina oxide powder and silicon diode as passive dosimeters [4]. Following irradiation, blood samples were maintained at 37º C for 2 hours allowing to act the cellular reparation mechanisms.

Cell culture. Lymphocytes were cultured for 48 hours in RPMI 1640 media (Life Technologies, Cergy Pontoise, France), supplemented with 20% (v/v) foetal calf serum, 1% (v/v) phytohemaglutinin (Life Technologies, Cergy Pontoise, France), 1% (v/v) Hepes, and 50 IU penicillin, 50 µg/ml streptomycin. Colcemide (Life Technologies, Cergy Pontoise, France) (0.05 µg/ml) was added 24 hours after cultures started and Calyculin A (Calbiochem, France, 50 nM) was added for the last hour. The cultured cells were treated with a hypotonic solution of KCl (0.075M) for 7 minutes at 37°C and fixed in three changes of fixative (methanol: acetic acid 3:1 v/v). Finally the fixed cells were dropped onto slides in a Thermotron equipment (VYSIS, France) with humidity controlled 45%, temperature 22°C and ventilation controlled. The slides were stained with Giemsa.

Scoring criteria. The incidence of cells having PCC cells into G1, G2 and M with more than 46 elements or 92 in late M phase were scored. Two operators participated to the scoring according the same criteria. A ring with a visible hole (with or without visible centromere) in any stage of cellular cycle and a couple of rings in partners (separated or united by the centromere) in metaphase cells was considered like one ring. When possible, at least 100 rings or 500 PCC cells for each radiation dose were scored. The PCC-R frequencies all stages of cellular cycle were evaluated as the ratios between rings scored and total observed cells. PCC-R were observed with a light microscope at x60 magnification (SA, Nikon, Japan). A Metasystem coupled to a microscope Nikon was used to take the pictures.

Statistics. The frequency Confidence interval was calculated assuming a Poisson distribution of aberrations in the cells. The u test was used to test whether dispersions of aberrations can be described by Poisson distribution [5]. When the distribution of aberration follows a Poisson statistical law, there is only 5% likelihood that the u value exceeds $|1.96|$. Another indicator of dispersion from Poisson distribution can be evaluated using the variance to mean ratio (σ^2/Y), since one characteristic of Poisson distribution is that the variance is equal to the mean. Comparison between means was made using student t test. Not significance differences were founded when calculated t values were lower than tabulated t values with a 95 % confidence. Dose-effect relationships were fitted according to a lineal quadratic model using the maximum likelihood method as described by Edwards [6] and Papworth,[7].

3. Results and Discussion

Figure 1 show some examples of Giemsa stained PCC cells induced by Calyculin A with PCC rings induced by cobalt 60 or neutrons irradiation. Calyculin A concentration (50 nM) was chosen according Kanda [8]. At this concentration the percentage of PCC cells among irradiated lymphocytes was high (around 20%), and the chromosomes were suitable for morphologic analysis. To higher Calyculin A concentrations the number of PCC cells is increased, but the yield of PCC rings falls probably because the over-treatment resulting in diffuse and short chromosomes which made chromosome analysis difficult [8].

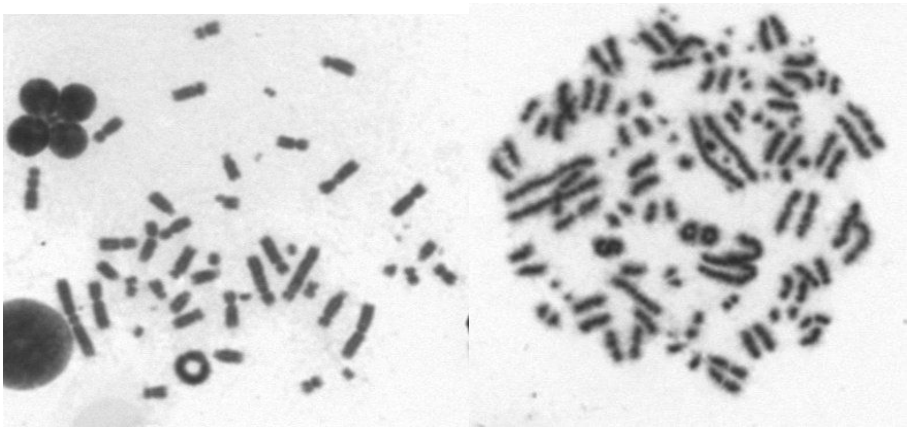

Figure 1 . Rings in PCC cells

Table 1 and Table 2 show the number of PCC cells scored, the frequency of PCC-R by dose and PCC-index expressed as a percentage of all nuclei observed.

Dose (Gy)	Number of scored cells	Number of PCC-R	PCC-R/cell Frequencies	PCC index
0	1000	0	0,00 ± 0,00	15.1%
5	1000	98	0,10 ± 0,02	12.2%
7,5	1100	168	0,15 ± 0,02	10.8 %
10	876	212	0,24 ± 0,03	7.8%
20	539	233	0,43 ± 0,04	6.0%
25	497	268	0,54 ± 0,04	4.3%

Table 1. Frequencies of PCC rings/cell and PCC index in lymphocytes exposed to different Co-60 doses

Gamma Dose (Gy)	Neutron dose (Gy)	Total dose (Gy)	Number of Scored cells	Number of PCC-R	PCC-R/cell Frequencies	PCC index
0	0	0	1000	0	0,00 ± 0,00	13.25%
0.7	4.7	5.4	1200	292	0,24 ± 0,02	6.4%
0.7	4.9	5.6	1025	296	0,29 ± 0,03	4.2%
1.0	8.4	9.4	972	317	0,33 ± 0,03	3.0 %
1.1	11.6	12.7	211	68	0,32 ± 0,06	0.54%
1.7	24.4	26.1	156	51	0,33 ± 0,07	0.51%

Table 2. Frequencies of PCC rings/cell and PCC index in lymphocytes exposed to different neutrons doses

PCC index decreased from 15.1 % to 4.3 % in Cobalt exposures and from 13.25 to 0.51 for neutron exposures. At 5 Gy Co-60 we obtain an PCC index of 12.2%, slightly lower than 17 % obtained by Kanda with 5 Gy X Rays [8] but very similar to Gotoh [2] who obtained an induction of G2 PCC cells around 11 % with 5 Gy of γ Rays.

A total of 9676 cells were analyzed, 5112 for Cobalt curve and 4564 for neutrons. For each curve two donors were analyzed. When comparing the PCC-R frequencies between donors we did not find significant differences to any ^{60}Co dose nor neutrons dose, for this reason, the curves were made with both donors together.

Table 3 and Table 4 show PCC-R distribution in lymphocytes exposed to different doses of ^{60}Co and neutrons respectively and associated U values.

Dose (Gy)	Cells scored	Number of PCC-R	Distribution					σ^2/X	"U"
			0	1	2	3	4		
0	1000	0	1000	0	0	0	0	-	-
5	1000	98	906	91	2	1	0	1.01	0.11
7.5	1100	168	947	139	13	1	0	1.04	1.04
10	876	212	689	161	24	1	1	1.06	1.26
20	539	233	426	166	40	6	1	1.06	1.07
25	497	268	343	107	41	5	1	1.18	2.84

Table 3. Distribution of PCC-R in lymphocytes exposed to different doses of ^{60}Co

Dose (Gy)	Cells scored	Number of PCC-R	Distribution					σ^2/X	"U"
			0	1	2	3	4		
0	1000	0	1000	0	0	0	0	-	-
5.4	1200	292	952	214	27	4	3	1.15	3.63
5.6	1025	296	773	214	32	6	0	1.05	1.14
9.4	972	317	714	209	40	8	1	1.12	2.57
12.7	211	68	153	49	8	1	0	1.01	0.06
26.1	156	51	114	34	7	1	0	1.07	0.64

Table 4. Distribution of PCC-R in lymphocytes exposed to different doses of neutrons

Rings distribution follows a Poisson for almost all cobalt and neutrons doses. Only for 25 Gy Cobalt curve and 9.4 Gy neutron curve the U values exceeded ± 1.96. We would have expected an overdispersion of rings for the high neutron doses which is not observed. However a saturation rings yield per cell is observed. Indeed if aberrations distribution are compared between all doses we have the same proportion of cells in each class. Comparison with the literature is difficult as most publications deals with dicentrics distributions and not rings. In addition for Cobalt exposure no dicentrics overdispersion is described in the literature above 5 Gy [9], however for fission neutron radiation literature is more conflictive [10]. In high LET radiations there is a high tendency to overdispersion of dicentrics [11].

The interest of distribution studies is to evaluate dose heterogeneity. Indeed when the irradiation is homogeneous a Poisson distribution of aberrations in cells is expected. It will be an interesting focus of this technique for its utility to evaluate individuals irradiated partially in small areas of the body. Although in radiations of high LET the overdispersion can mask partial irradiations [11]. But referring to Kanda [1], we have less information about ring distribution than dicentrics one [12].

Figure 2 shows dose-effect relationship for the *in vitro* induction of PCC-R in lymphocytes to different ^{60}Co doses. The PCC-R frequencies were fitted to doses and fitted values of the C and α coefficients of lineal function are presented on the graphic. The curve shape is different from Kanda [1] as they observe a three modal curve, one lineal part form 0 to 10 Gy followed by a small saturation phase from 10 to 20 Gy and finally a plateau phase from 20 to 40 Gy.

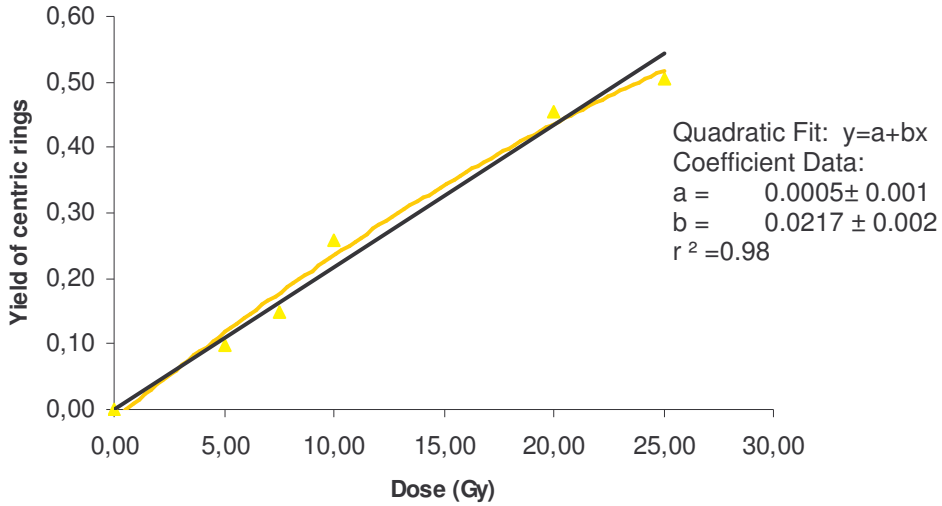

Figure 2. Relationship between PCC-R frequencies in lymphocytes and different ^{60}Co doses

The PCC-R frequencies obtained in this work for ^{60}Co and neutrons dose radiation are 5 times lower than those obtained by Kanda for X Rays [1], even if our scoring criteria where carefully selected to match the one described in Kanda's report [1]. This variation may be due to the number of cells analyzed, type of radiation, dose rate employed, score criteria, and in general to the culture condition employed. Therefore, it's important to realize the dose estimation in similar

culture conditions employed in dose effect curves. By this way, variations on the dose are reduced [13].

Figure 3 shows dose-effect relationship for the induction *in vitro* induction of PCC-R in lymphocytes to different doses of neutrons. The observed PCC-R frequencies had a lineal increase with the doses up to 10 Gy, and then saturation was observed. The data of the lineal part was fitted to a lineal model with values of b coefficient of 0.042. Apparently the saturation of PCC rings curves can occur to different doses for different kinds of radiation. In our work saturation started from 10 Gy neutron radiation (Fig 3 and Fig. 4), whereas our results and Kanda's data suggest saturation starting from 20 Gy for Gamma and X-rays [1]. Here again there is no other PCC-curves for neutrons but it is possible to compare the yield of PCC-R with yield of centric rings score in the conventional cytogenetic technique. An article published by Lloyd shows centric rings rates for different neutron configurations [14]. For a 3 Gy dose of 0.7 Mev neutron radiation, the rate of centric ring per cell is 17/37 = 0.46 which is two times higher than the 5 Gy experiment presented here [14]. But we have no data for doses below 5 Gy.

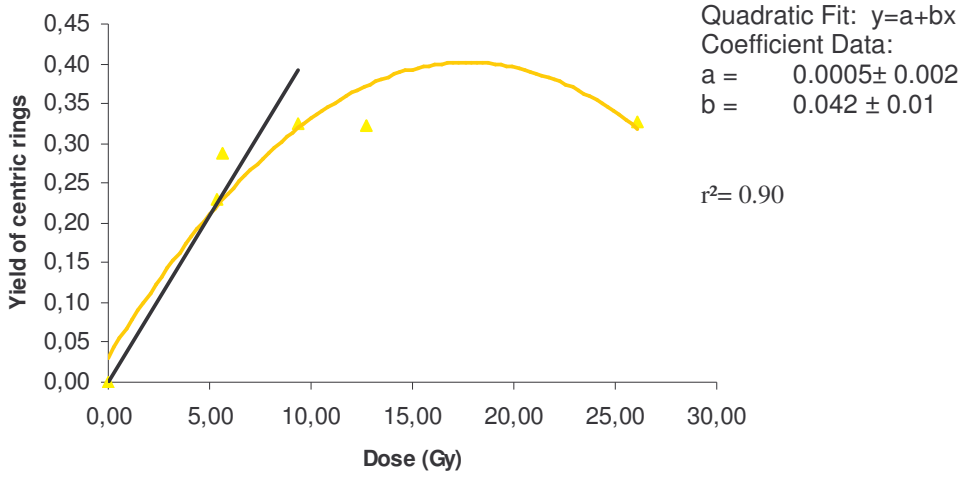

Figure 3. Relationship between PCC-R frequencies in lymphocytes and different doses of neutrons

Such curves are useful in case of accidental overexposure to high doses as in the Tokai-mura accident. It was the first case where this technique was used to estimate a dose in a real accident. PCC-R yield in a patient were measured and equivalent gamma doses were estimated using X-rays (200kV) curve published by Kanda [1]. However, exposure was mostly due to neutron radiations but such curves were not available at that time. If the curves presented in this publication are used to estimate dose received by these victims, doses estimated become: 18 Gy for patient B and 5,7 for patient C. It is not possible to estimate a dose for patient A as the yield of PCC-R is too high, out of the curve linear range. These doses are higher than the equivalent gamma dose measured by Hayata [15] which were 7.4 Gy for patient B and 2.3 Gy for patient C. Such a result in unexpected as the neutron dose should be lower than the gamma dose because the Relative Biological Effect of neutrons is around 10 times higher than for X-rays. This unexpected result is linked to our neutron dose effect curve which is lower than Kanda X –ray curve [1].

4. CONCLUSIONS

This technique is suitable to estimate high doses of radiation up to 25 Gy of gamma irradiation and up to 10 Gy of neutrons. It has the advantage to be able to estimate high radiation doses. The addition of Calyculin increases the mitotic index of cell cultures. This inhibitor of protein phosphatase can also be used to increase to number of analyzable cells in conventional cytogenetic if only cells at the metaphase stage are analyzed.

5. ACKNOWLEDGMENTS

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