



HAL
open science

Biodosimetry estimation using the ratio of the longestshortest length in the premature chromosome condensation (PCC) method applying autocapture and automatic image analysis

J.E. González, I. Romero, Eric Gregoire, C. Martin, A.I. Lamadrid, P. Voisin, J.-F. Barquinero, O. García

► To cite this version:

J.E. González, I. Romero, Eric Gregoire, C. Martin, A.I. Lamadrid, et al.. Biodosimetry estimation using the ratio of the longestshortest length in the premature chromosome condensation (PCC) method applying autocapture and automatic image analysis. *Journal of Radiation Research*, 2014, 55 (5), pp.862-865. 10.1093/jrr/rru030 . hal-02641714

HAL Id: hal-02641714

<https://hal.science/hal-02641714v1>

Submitted on 23 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Biodosimetry estimation using the ratio of the longest:shortest length in the premature chromosome condensation (PCC) method applying autocapture and automatic image analysis

Jorge E. GONZÁLEZ^{1,†*}, Ivonne ROMERO^{1,†}, Eric GREGOIRE², Cécile MARTIN², Ana I. LAMADRID¹, Philippe VOISIN², Joan-Francesc BARQUINERO² and Omar GARCÍA¹

¹Centro de Protección e Higiene de las Radiaciones, Calle 20 No. 4113, e/41 y 47, Playa, CP 11300, La Habana, Cuba

²Institut de Radioprotection et de Sûreté Nucléaire, BP 17, 92262 Fontenay-aux-Roses, France

*Corresponding author. Centro de Protección e Higiene de las Radiaciones, Calle 20 No. 4113, e/41 y 47, Playa, CP 11300, La Habana, Cuba. Tel: +53-7-682-9571; Fax: +53-7 682-9573; Email: jorgee@cphr.edu.cu

[†]Both authors contributed equally to the work.

(Received 5 December 2013; revised 26 March 2014; accepted 29 March 2014)

The combination of automatic image acquisition and automatic image analysis of premature chromosome condensation (PCC) spreads was tested as a rapid biodosimeter protocol. Human peripheral lymphocytes were irradiated with ⁶⁰Co gamma rays in a single dose of between 1 and 20 Gy, stimulated with phytohaemagglutinin and incubated for 48 h, division blocked with Colcemid, and PCC-induced by Calyculin A. Images of chromosome spreads were captured and analysed automatically by combining the Metafer 4 and CellProfiler platforms. Automatic measurement of chromosome lengths allows the calculation of the length ratio (LR) of the longest and the shortest piece that can be used for dose estimation since this ratio is correlated with ionizing radiation dose. The LR of the longest and the shortest chromosome pieces showed the best goodness-of-fit to a linear model in the dose interval tested. The application of the automatic analysis increases the potential use of the PCC method for triage in the event of massive radiation casualties.

Keywords: PCC assay; image analysis; high-dose radiation; Calyculin A

INTRODUCTION

Chemically induced premature chromosome condensation (PCC) methods have been suggested as a useful tool in biodosimetry in cases of accidental exposure [1–5].

The number of extra chromosomal pieces in G2-PCC correlates well with radiation dose and has been proposed for estimating radiation doses occurring in emergencies and accidents [6]. Nevertheless, scoring requires the selection of G2-PCC cells from all the PCC spreads, and at high doses the number of induced fragments is too large for reliable visual scoring. Ring chromosomes have also been proposed as a biological endpoint to be analysed in PCC cells. They are induced at a much lower frequency than fragments, and this makes ring scoring a feasible endpoint after a very high dose. However, ring identification requires precise criteria

that may differ between labs and is a difficult endpoint for automatic identification; even so, it is actually the most popular of the chemically induced PCC methods used in biological dosimetry [1].

In 2005, Gotoh and Tanno presented the length ratio (LR) between the longest and the shortest chromosomal piece as an alternative method for a rapid dose estimate in the case of a radiation accident [6]. In analysing PCC-cells, Gotoh and Tanno noted an increase in unusually long chromosome pieces in cells exposed to high doses of ionizing radiation. They measured their lengths and calculated the LR, and as expected the LR increased with radiation in a dose-dependent manner. The same method applied by Wang and co-workers for biodosimetry of high-LET ionizing radiation [7]. Until now, image capture and measuring of chromosome length required the presence of an operator in all steps of a

semi-automatic conducted process. The aim of the present study was to evaluate the feasibility of combining automatic image acquisition and automatic image analysis to calculate the LR of the longest and the shortest chromosomal pieces.

MATERIALS AND METHODS

Blood irradiation

Peripheral blood samples from three healthy donors were obtained with informed consent according to the institutional ethical procedures of IRSN (Fontenay aux Roses, Paris). Samples were exposed to doses of 0, 1, 2, 5, 7.5, 10, 15 and 20 Gy (dose-rate 0.5 Gy/min) using a ^{60}Co source of gamma radiation at the metrology laboratory of IRSN. The IAEA recommendations for cytogenetic dosimetry were followed during irradiations [1]. Briefly, blood samples were located inside a plexiglass holder that was submerged in a water bath heated to 37°C and placed in front of a ^{60}Co gamma ray source during irradiation.

Lymphocyte culture and PCC assay

The PCC chemically induced assay was conducted as described previously [8]. In all cases, 0.5 ml of peripheral whole blood was cultured for 48 h in 5 ml of RPMI 1640 medium containing L-glutamine, 20% fetal calf serum and 1% phytohaemagglutinin (PHA). Colcemid (0.05 µg/ml) was added 24 h after the beginning of the culture, and Calyculin A (50 nM) was added 1 h before the harvest. Cultured cells were treated with a hypotonic solution of KCl (0.075M) for 8 min at 37°C and fixed in three changes of fixative (methanol:acetic acid, 3:1 v/v). Finally, 30 µl of the resulting cell suspension was dropped onto slides, air-dried and stained with 4% Giemsa solution.

Automatic capture and image analysis

Digitalized images at $\times 63$ magnification were obtained automatically with a motorized AxioImager Z1 microscope (Carl Zeiss, Oberkochen, Germany) coupled to an AxioCam HRm camera (Carl Zeiss MicroImaging, GmbH, Jena, Germany) controlled by image analysis program Metafer 4 with MSearch and transmitted light (TL) mode. Images from the Metafer 4 gallery that had apparently complete sets of chromosomal pieces and no overlapped chromosomes were selected by an operator for analysis. A total of 25 images per subject were selected, since no statistically significant change was found in LR ratio by further increasing the number of images analysed. The images were saved as black and white images in TIF format. For image analysis, CellProfiler 2.0 (revision: 10997) software package for Windows was obtained from <http://www.cellprofiler.org> 19 March 2013, date last accessed [9]. The software uses a pipeline (available upon request from the authors) of modules designed to automatically identify, quantify and export the area–shape measurements of chromosome pieces with all

PCC spreads analysed. For the CellProfiler pipeline setting, the semiautomatic (by manual drawing) and automatic measurements were compared using Bland–Altman analysis, and a bias of -0.3 pixels with a confidence interval from -0.9 to 0.3 was obtained, which was considered negligible.

From all the area–shape measurements, the major axis length was taken into consideration and the data processed to obtain the minimum and maximum length value per spread analysed. The maximum and minimum values were divided to obtain the LR. Considering the absolute chromosome length varies depending on the degree of chromosome condensation of each chromosome spread, the LR of the longest: shortest chromosome was calculated from each individual spread. The LRs between G2 and metaphase spreads were not statistically different ($P > 0.05$); consequently both types of spreads were analysed.

The pipeline also saves a resultant image with each chromosome piece detected (outlined in color) and the measurement of the major axis length of the chromosome pieces indicated as a quality control step (Fig. 1). The analysis was performed with an i3 Intel processor and 4 GB of RAM running on Windows 7.

Statistical analysis

The slope and the intercept of the dose–response linear regression were estimated with functional relationship by the maximum likelihood method considering in the model the mean value and the error calculated from the individual data of the three donors. The significance of the slope and the intercept were evaluated by *t*-test and the goodness-of-fit by chi-square test. The difference between donors was tested using the *F* test for comparing lines. The alpha level assumed for all tests was 0.05.

RESULTS

Figure 1 shows the Giemsa-stained chromosomal spreads obtained by the PCC method after exposure to low-LET ionizing radiation as analysed by the CellProfiler pipeline. Small pieces of chromosomes and especially long chromosome pieces are increased with ionizing radiation dose (Fig. 1B). The LR increased linearly with the radiation dose up to the dose of 20 Gy, the highest tested (Fig. 2). The dose–response of the three subjects (Table 1) were compared by *F* test and the datasets obtained were not statistically different. The variability between subjects represented by the coefficient of variation ranged from a minimum of 2% at 2 Gy dose to a maximum of 21% for non-irradiated (0 Gy) samples (Table 1). The slope of the line fitted (Fig. 2) from the mean dataset was 0.61 Gy^{-1} with 0.05 Gy^{-1} of error, and the intercept was 5.60 with an error of 0.21.

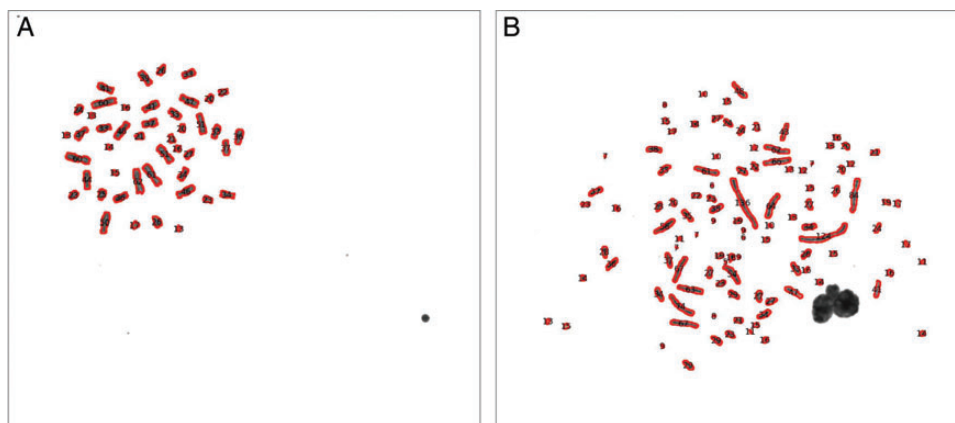


Fig. 1. Images of chromosomal spreads from cultures stimulated with phytohaemagglutinin and incubated for 48 h; the division was blocked with Colcemid and premature chromosome condensation (PCC) was induced by Calyculin A. The images were autocaptured at $\times 63$ magnification by Metafer 4. (A) Image from a control (0 Gy) sample with the chromosome pieces detected (outlined in red) and measured. (B) Image from an irradiated (20 Gy) sample with all the chromosome pieces detected (outlined in red) and measured.

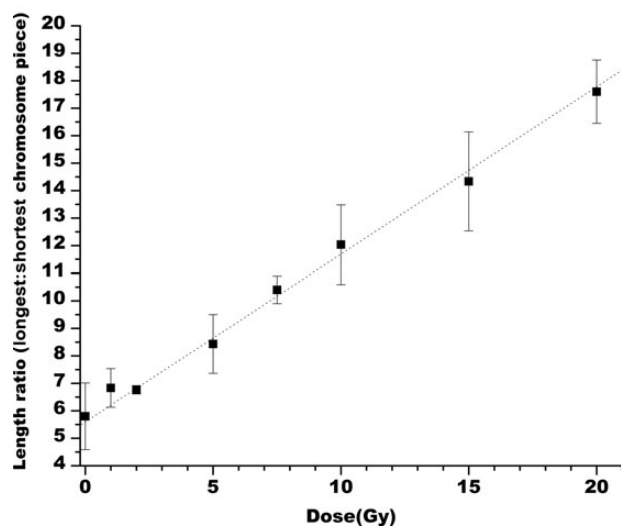


Fig. 2. Linear dose–response of the length ratio (LR) of the longest:shortest chromosome pieces per spread. The mean (symbols) of the three subjects with their standard deviations (error bars) were used in the mathematical model for the fit. The alpha coefficient was 0.61 ± 0.05 , and the intercept was 5.60 ± 0.21 .

DISCUSSION

After radiation exposure accidents the absorbed dose should be determined as quickly as possible; this is particularly critical in a mass casualty scenario. The cytogenetic approaches actually used for biological dosimetry are time consuming (particularly the microscopic analysis), and scorer variability is also a handicap. Automation is a way to overcome these limitations. Attempts have been made to automate

Table 1. Length ratio (LR) of the longest:shortest chromosome pieces per dose point from the three subjects analysed

Dose (Gy)	Length ratio (LR) of the longest:shortest chromosome pieces ^a			Mean \pm SD	CV (%)
	Donor 1	Donor 2	Donor 3		
0	4.4	6.5	6.5	5.8 ± 1.2	21
1	6.1	7.5	6.9	6.8 ± 0.7	10
2	6.9	6.8	6.6	6.8 ± 0.2	2
5	7.2	9.1	9.0	8.4 ± 1.1	13
7.5	9.9	10.9	10.4	10.4 ± 0.5	5
10	11.1	13.7	11.3	12.0 ± 1.4	12
15	12.5	16.1	14.4	14.3 ± 1.8	13
20	17.2	18.9	16.7	17.6 ± 1.2	7

^a Mean value of 25 spreads. The means and their standard deviations were used to calculate the coefficients of variation (CVs).

scoring for dicentric and micronuclei assays [1]. The automated metaphase finding on Giemsa-stained preparations has been used in PCC assay, followed by operator scoring by eye [10], but no further attempt has been reported for the full automatic analysis of PCC spreads.

Here we tested a simple and potentially useful biodosimetry protocol based on calculating the LR of the longest and the shortest chromosome pieces together with automatic capture and analysis of the PCC spreads. The inclusion of

two automatic steps in the analysis extends previous work [6, 7]; semiautomatic drawing and the method of measurement using Image J software increases the potential usefulness of this approach, especially when a large number of samples are received simultaneously. The analysis can be conducted without the need for special training in cytogenetics (eg. dicentric or small ring recognition) and should be useful for rapid and massive dose estimates in cases of accidents potentially involving high levels of ionizing radiation. An exercise with different laboratories participating is desirable in order to test the speed of response and to evaluate the variability in results.

Understanding the *in vitro* dose–response relationship is a crucial step for the applicability of biological dosimeters. It allows the use of the proposed methodology in real situations. At the background level, we obtained a value for LR of 5.8 ± 1.2 , quite similar to the reported value of 5 [6]. Previously pooled LR data from subject samples [6] were used for fitting data based on cytophotometry studies that established a quite constant length for chromosomes 1 (longest) and 22 (shortest) among individuals at the background level i.e. not exposed to radiation [11]. However, this was performed after normalization of the length by linear regression of the measured DNA content of chromosomes 2, 3 and 4 against the expected normalized DNA content and could be different for direct measurements without normalization [11]. Gotoh and Tanno [6] used the direct measurement of the chromosome pieces and assumed a nearly constant LR value at the background level without further consideration of individual data. Here we decided to combine the direct measurement of the chromosome pieces with the consideration of individual variability, and consequently we used the means of the three donors with their standard deviations for fitting a dose–response curve. The best fit was obtained with the lineal model. Several useful mathematical models (i.e. square root, power and exponential rise to maximum) have previously been fitted to the LR data after low-LET radiation with an acceptable goodness-of-fit [6]. The reason for the apparent contradiction between the linear model obtained here and these reported models is, presumably, the dose interval used. The LR data show apparent saturation after doses > 20 Gy according to the graphic information presented in previous reports [6, 7]. According to the same graphic information, the LR data increase, presumably linearly, with doses up to 20 Gy.

The possibility of establishing a dose–response relationship by automatic image capture and analysis of PCC spreads confirms the utility of this approach for high-dose biodosimetry. This procedure increases the potential use of the PCC method for triage in case of massive radiation casualties and should be considered by the biodosimetric community as a promising tool with which to complement the dicentric assay.

ACKNOWLEDGEMENTS

Special thanks to Gordon Livingston for editorial assistance and helpful comments.

FUNDING

This work was supported by a grant from the International Atomic Energy Agency (CUB/10010) awarded to I.R from Project RLA/9074. Funding to pay the Open Access publication charges for this article was provided by IRSN (Fontenay-aux-Roses, France).

REFERENCES

1. IAEA (ed). *Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. EPR-Biodosimetry*. Vienna: International Atomic Energy Agency, 2011.
2. Gotoh E, Tanno Y, Takakura K. Simple biodosimetry method for use in cases of high-dose radiation exposure that scores the chromosome number of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). *Int J Radiat Biol* 2005;**81**:33–40.
3. Kanda R, Hayata I, Lloyd D-C. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. *Int J Radiat Biol* 1999;**75**: 441–6.
4. Lamadrid A-I, García O, Delbos M *et al*. PCC-ring induction in human lymphocytes exposed to gamma and neutron irradiation. *J Radiat Res* 2007;**48**:1–6.
5. Lindholm C, Stricklin D, Jaworska A *et al*. Premature chromosome condensation (PCC) assay for dose assessment in mass casualty accidents. *Radiat Res* 2010;**173**:71–8.
6. Gotoh E, Tanno Y. Simple biodosimetry method for cases of high-dose radiation exposure using the ratio of the longest/shortest length of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). *Int J Radiat Biol* 2005;**81**: 379–85.
7. Wang Z-Z, Li W-J, Zhi D-J *et al*. Biodosimetry estimate for high-LET irradiation. *Radiat Environ Biophys* 2007;**46**: 229–35.
8. Romero I, García O, Lamadrid A-I *et al*. Assessment of simulated high-dose partial-body irradiation by PCC-R assay. *J Rad Res* 2013;**54**:863–71.
9. Carpenter A-E, Jones T-R, Lamprecht M-R *et al*. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* 2006;**7**: R100.
10. Blakely W-F, Prasanna P-G-S, Kolanko C-J *et al*. Application of the premature chromosome condensation assay in simulated partial-body radiation exposures: evaluation of the use of an automated metaphase-finder. *Stem Cells* 1995;**13** Suppl 1: S223–30.
11. Groen F-C, Van der Ploeg M. DNA cytophotometry of human chromosomes. *J Histochem Cytochem* 1979;**27**:436–40.