大韓獸醫學會誌(1993) 第33券 第3號
Korean J Vet Res (1993) 33(3) : 487~492

The cytokinesis-block micronucleus assay as a biological dosimeter in irradiated lymphocyte : Comparison of the response of mouse and human

Sung-ho Kim*, Chul-koo Cho**, Tae-hwan Kim*, Seong-yul Yoo**, Kyoung-hwan koh**, Hyong-geun Yun**, Joo-hwan Koh*, Soo-yong Choi***
Laboratory of Radiation Medicine, Depatmenmt of Therapeutic Radiology**, Laboratory of Epidemiology, Korea Cancer Center Hospital****
(Received July 2, 1993)

임파구의 미세핵 분석법을 이용한 생물학적 방사선 피폭선량 측정 : 마우스와 인체에서의 반응비교

김성호* · 조철구** · 김태환* · 류성렬* · 고경환**
윤형근** · 고주환* · 최수용***
한국원자력병원 방사선의학연구실*, 치료방사선과** 역학연구실***
(1993년 7월 2일 접수)

초록 : 정상인 말초혈액임파구 및 C57BL/6마우스 비장임파구에 ^{60}Co γ-rays를 *in vitro* 상태에서 조사한 후 500개 또는 1000개의 cytokinesis-blocked(CB) lymphocytes의 미세핵(micronuclei)의 발생빈도를 측정하였다. 방사선조사량에 따라 미세핵의 발생빈도는 증가하였으며 linear-quadratic model로 측정한 결과 선량반응곡선의 식은 인체의 경우 $Y=(0.31\pm0.049)D+(0.0022\pm0.0002)D^2+13.19(r^2=1.000)$이었으며, 마우스의 경우 $Y=(1.31\pm0.264)D+(0.0015\pm0.0006)+8.7(r^2=0.988)$이었다(Y는 1000개의 CB cell 당 미세핵발생빈도, D는 cGy로 표시되는 조사선량). 인체 말초혈액임파구에 대한 마우스 비장임파구의 상대적 생물학적 효과(relative biological effectiveness)는 미세핵의 발생율이 세포당 0.05~0.8의 범위에서 1.84±0.48이었다. 미세핵분석법은 인체 및 동물의 방사선 피폭시 간편하고 빠른 생물학적 선량측정법으로 사용될 수 있을 것이다.

Key words : micronuclei, biological dosimetry, cytokinesis blocked cell, mouse, relative biological effectiveness.

Introduction

Measurement of radiation response by simple and informative techniques would be of great value in studying genetic risk following occupational, therapeutic or accidental exposure to radiation. Biological dosimetry has a number of applications.[1] The most obvious one is in cases of radiation accidents with a lack of physical dosimetry. Sometimes physical dosimetric methods must be supplemented by biological assays, for example after partial-body exposure with the physical dosimeter outside the radiation field.

One of the biological methods adopted for dosimetry purposes, cytogenetic analysis has been the most popular one.[1,2] The occurrence of chromosome aberrations in peripheral blood lymphocytes(PBLs) has been used.

Although this is a sensitive method for dose estimation, it is laborious. An alternative and simple cytogenetic technique is the measurement of MN frequency in cultured human lymphocytes.[3] Compared to the classical cytogenetic methods for evaluating chromosomal damage[4], The MN assay for PBLs is relatively simple and allows a rapid scoring of a large number of cells by personnel not specially trained for chromosomal analysis.[1]

The present study was performed to study micronucleus induction in human PBLs and mouse spleen lymphocytes(SLs) treated with γ -rays or neutrons and to determine the RBE between mouse and human.

Materials and Methods

Cell culture : PBLs and SLs were seperated from whole blood of four healthy men and three C57BL/6 mice spleen suspension on Ficoll-Hypaque gradients, washed twice in Hank's balanced salt solution and resuspended in RPMI 1640 medium(GIBCO, Grand Island, NY) containing Hepes buffer, 15% heat inactivated foetal calf serum, L-glutamine and antibiotics. The lymphocytes were cultured in multi-well tissue culture plates(Corning, No. 25820, NY) at concentration of 5×10^5 cells/mℓ. An optimum concentration of phytohaemagglutinin(PHA, 5 μg/mℓ, Sigma, St. Louis, Mo) or *Concanavalin* A(Con A, 2.5 μg/mℓ, Signa, St. Louis, Mo) was used to stimulate the lymphocytes to transform and divide in culture. The cells were cultured at 37℃ in a humidified atmosphere containing 5% CO_2.

Irradiation : One sample served as a control for determining the spontaneous MN frequency. The others were irradiated with 124.8, 186.8, 280.4 or 395.6 cGy for human PBLs and 100, 200, 300, 400, 500 for mouse SLs of ^{60}Co γ -rays(Theratron-780 teletherapy unit) at a rate of 211 cGy/min in a 37℃ water bath, respectively. The doses were measured with Capintec PR-06C farmer type chamber and Capintec 192 electrometer(Capintec, USA).

Cytokinesis-block method : Cytochalasin B(Cyt-B, Aldrich Chemical Co., west Saint Paul)was maded up as a stock solution in dimethylsulphoxide at a concentration of 2 mg/mℓ divided in small portions and stored at -70℃

The stock solution of Cyt-B was thawed, diluted in medium and added 44h, for human PBLs, or 21h, for mouse SLs, after commencement of the culture at a concentration of 3.0 μg/mℓ, After an incubation period of 72h, for human PBLs, or 41h, for mouse SLs, the cells were collected by centrifugation and resuspended in a mixture of methanol : gracial acetic acid(3 : 1). The fixed cells were transfered to a slide, air-dried and stained with 10% Giemsa for 10 min.

Scoring of micronuclei : The MN were scored in 500-1000 binucleated CB cells using a 400× magnification. For the identification of MN published criteria were applied.[5] Examples of CB cells with different frequency number of MN are showen Fig 1.

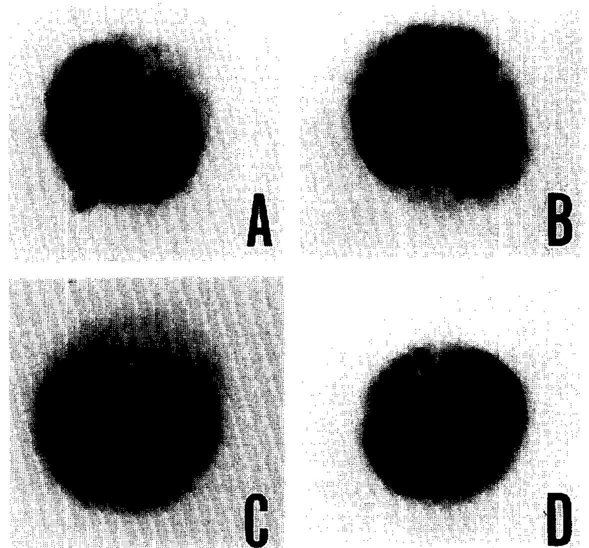

Fig 1. Cytokinesis-blocked cells without a MN (A), with 1 MN (B), 2 MN (C), and 3 MN (D).

Results

The data obtained in the dose-response study are summrized in Table 1 and 2. The MN frequency in unexposed lymphocytes was not significantly different from donor to donor. The baseline number of MN per CB cell in unirradiated lymphcytes was very low(Table 3). Fig 2 and 3 show the results for individual donors, the curves obtained from the pooled data of donors and best fitting linear-quadratic curves, respectively. There was a significant relationship between the frequency of induced MN and dose of γ -rays (human : $r^2 = 1.000$)(mouse : $r^2 = 0.988$). When analysed by linear-quadratic model the line of best fit was:

human : $y = (0.31 \pm 0.049)D + (0.0022 \pm 0.0002)D^2 + 13.19$

Table 1. Micronuclei(MN) per 1000 cytokinesis blocked(CB) cells for the individual donors after γ-rays exposure

Dose (cGy)	Number of cells without MN	Frequency distribution of the number of within one CB cell							Total number of MN
		1	2	3	4	5	6	7	
Donor1 :	Male, 29y								
0	989	10	1						12
124.8	932	61	7						75
186.8	866	115	18			1			156
280.4	799	159	35	7					250
395.6	676	210	91	19	4				465
Donor2 :	Male, 28y								
0	990	8	2						12
124.8	920	69	10	1					92
186.8	887	113	8	1	1				136
280.4	792	166	33	6	2	1			263
395.6	639	241	81	27	8	3		1	538
Donor 3 :	Male, 18y								
0	989	9	2						13
124.8	910	76	12	2					106
186.8	864	125	9	2					149
280.4	787	174	29	10					262
395.6	661	220	94	20	5				488
Donor 4 :	Male, 33y								
0	987	12	1						14
124.8	914	79	5	2					95
186.8	878	107	13	2					139
280.4	755	189	44	12					313
395.6	688	185	100	23	3			1	473

Table 2. Micronuclei(MN) per 500 cytokinesis blocked(CB) mouse lymphocytes after γ-rays exposure

Dose (cGy)	Number of cells without MN	Frequency distribution of the number					Total number of MN
		1	2	3	4	5	
0	495	5					5
	496	4					4
	496	4					4
100	456	38	4	2			52
	448	46	6				58
	458	39	3				45
200	376	99	22	3			152
	371	112	17				146
	383	98	19				136
300	297	151	41	8	2	1	270
	300	134	50	16			282
	258	162	69	11			333
400	200	213	72	11	4		406
	231	167	79	19	4		398
	224	181	71	24			395
500	165	199	103	28	5		509
	177	183	120	15	5		488
	161	203	108	24	4		507

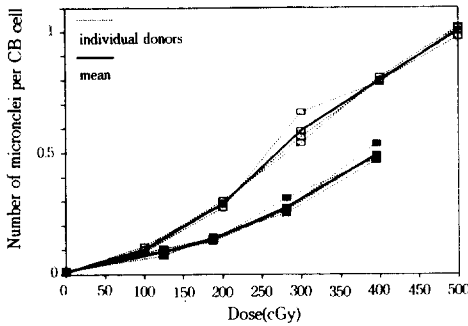

Fig 2. The dose-response relationship of micronuclei in binucleated human(■) or mouse(□)lymphocytes following treatment with r-rays.

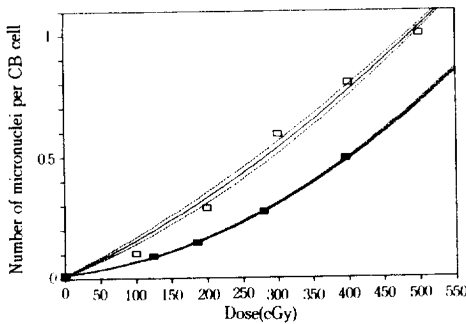

Fig 3. Dose-reponse for r-rays induced micronuclei in human(■) and mouse(□)lymphocytes. The solid and dashed lines reporesent the results of a linear-quadratic fit throught the data indicated in the figure.

mouse : $y=(1.31\pm0.264)+(0.0015\pm0.0006)D^2+8.7$

where y=number of MN/1000 CB cells and D=irradiation dose in cGy.

In order to determine the RBE of mouse SLs compared with human PBLs, the equation of $y=aD+bD^2+c$ was transformed as

$$D=\frac{[-a\pm\sqrt{a^2-4b(c-y)}]}{2b}$$

The RBE of mouse SLs to human PBLs was obtained from this equation. In the MN frequency between 0.05 and 0,8 per cell, the RBE of mouse was 1.84 ±0.48(Table 4).

Discussion

The mutagenic and carcinogenic risk associated with exposure to ionizing radiation has stimulated considerable interest in measuring genetic alteration in human cells. Analysis of MN in lymphocytes is a simpler and faster method for measuring chromosome damage. However, MN originate from acentric fragments of whole chromosomes, and this provide a measure of both chromosome breakage and loss, which is a somewhat different spectrum of damage from that obtained by chromosome analysis. Enumeration of MN in CB cells allows chromosome damage to be analysed in lymphocytes which have divided once only. This can be achieved in metaphase analysis only if bromodeoxyuridine uptake and differential staining is included in the protocol, to distinguish between first and second division metaph-

Table 3. Frequency of micronuclei in binucleated lymphocytes following treatment with γ-rays

Dose(cGy)	Micronuclei per cell(M±SE)
Human	
0	0.013±0.0002
124.8	0.092±0.0032
186.8	0.145±0.0023
280.4	0.272±0.0070
395.6	0.491±0.0082
Mouse	
0	0.009±0.0008
100	0.103±0.0092
200	0.289±0.0114
300	0.59 ±0.0473
400	0.799±0.0080
500	1.003±0.0164

Table 4. Relative biological effectiveness(RBE) of micronuclei(MN) induction between human and mouse lymphocytes following treatment with γ-rays

MN per cell	Human dose(Dh) required(cGy)*	Mouse dose(Dm) required(cGy)*	RBE (Dh/Dm)
0.05	76.29±2.09	30.67±2.75	2.49±0.23
0.1	139.48±2.59	65.17±4.75	2.14±0.16
0.2	228.49±2.76	127.79±7.04	1.79±0.10
0.4	353.8 ±2.95	235.82±8.30	1.50±0.05
0.8	530.72±3.08	412.12±5.95	1.29±0.02

* Calculated from fitting linear-quadratic model.

ases. The CB method has already been shown to be simple, reliable and above all very sensitive[6~7] as a result of the statistical power afford by the high scoring rate achievable(usually 1000 CB cells/30 min). The technique does not require highly specialized staff and should therefore be readily implemented for routine dosimetry.[10~13] Furthermore automated scoring of MN should be relatively simpler than automated metaphase analysis and image analysis systems are being developed for this purpose.[14~15]

MN frequency of two species gave a good fit to linear-quadratic model. The results of this experiment show that mouse is twice as sensitive as the human to the induction of MN at low dose level. We assume that the reason of this difference is concerned with chromosome arm number. Mouse, with an effective arm number 40, had twice as many MNs as the human, with as effective arm number 81. It is suggested that the chromosome arm number of a species influences the yield of MN. Several recent technical developments may further enhance the use of the CB method for *in vitro* dosimetry. Identification of kinetochores within MN using anti-kinetochore antibodies[16] or centromeres using centromeric probes[17] provides a means of distinguishing MN containing whole chromosomes from MN containing acentric fragments. thus providing better definition of the endpoint scored.

The ideal biological dosimeter should be rapid, easy and all the different radiation qualities should be covered by method(1). From this viewpoint, the CB MN assay may have the potential to complement metaphase analysis of chromosomes for estimating chromosome damage in human or animal lymphocytes following *in vivo* irradiation. Automation of the CB MN technique and dicentric chromosome analysis are real possibilities that would enhance the combined application of these methods for population monitoring.

Conclusion

The dose response of the number of micronuclei in cytokinesis-blocked(CB) lymphocytes after *in vitro* irradiation with γ-rays in the several dose ranges was studied for the mouse and human. One thousand or five hundred binucleated cells were systematically scored for micronucles. Measurements performed after irradiation showed a dose-related increase in micronuclei(MN) frequency in each of the donors studied. The dose-responses curves were analyzed by a linear-quadratic model, frequencies per 1000 CB cells were$(0.31 \pm 0.049)D + (0.0022 \pm 0.0002)D2 + 13.19(r^2 = 1.000)$ in human peripheral blood lymphocytes(PBLs), and $(1.31 \pm 0.264)D + (0.0015 \pm 0.0006)D2 + 8.7(r2 = 0.988)$ in mouse spleen lymphocytes(SLs) (D is irradiation dose in cGy). The relative biological effectiveness (RBE) of mouse LSs compared with human PBLs was estimated by best fitting linear-quadratic model. In the micronuclei frequency between 0.05 and 0.8 per cell, the RBE of mouse SLs was 1.84 ± 0.48. Since the MN assay is simple and rapid, it may be a good tool for evaluating the radiation response in human and animal.

References

1. Muller WU, Streffer C. Biological indicators for radiation damage. *Int J Radiat Biol* 1991 ; 59 : 863~873.
2. Lloyd DC. An overview of radiation dosimetry by conventional cytogenetic methods. In : Eisert WG and Mendelsohn ML, ed. *Biological dosemetry*. Sprinr-Verlag Press, 1984 ; 3~13.
3. Countryman PI, Heddle JA. The production of micronuclei from chromosome aberration in irradiated cultures of human lymphocytes. *Mutation Res* 1976 ; 41 : 321~332.
4. IAEA, International Atomic Energy Agency : Biological dosimetry : chromosomal aberration analysis for dose assessment. Technical report 260. Vienna, IAEA publications. Vienna, 1986.
5. Almassy Z, Krepinsky AB, Bianci A, et al. The present state and perspectives of micronucleus assay in radiation protection. A review. *Appl Radiat Isot* 1987 ; 38 : 241~249.
6. Gantenberg HW, Wuttke K, Streffer C, et al. Micronuclei in human lymphocytes irradiated *in vitor* or *in vivo*. *Radiat Res* 1991 ; 128 : 276~281.
7. Kormos C, Koteles GJ. Micronuclei in X-irradiated human lymphocytes. *Mutation Res* 1988 ; 199 : 31~35.
8. Ramalho A, Sunjevaric I, Natarajan AT. Use of frequencies of micronuclei as quantitative indicators of X-ray-induced chromosome aberrations in human p-

eripheral blood lymphocytes : comparison of two methods. *Mutation Res* 1988 ; 207 : 141~146.

9. Vral A, Thierens H, de Ridder L. Study of dose-rate and split-dose effects on the *in vitro* micronucleus yield in human lymphocytes exposed to X-rays. *Int J radiat Biol* 1992 ; 61 : 777~784.
10. Hall SC, Wells J. Micronuclei in human lymphocytes as a biological dosemeter : preliminary data following beta irradiation *in vitro, J Radiol Prot* 1988 ; 8 : 97~102.
11. Hubber R, Braselmann H, Bauchinger M. Intra-and inter-individual varation of background and radiation-induced micronucleus frequencies in human lymphocytes. *Int J Radiat Biol* 1992 ; 61 : 655~661.
12. Prosser JS, Moquet JE, Lloyd DC, et al. Radiation induction of micronuclei in human lymphocytes. *Mutation Res* 1988 ; 199 : 37~45.
13. Thierens H, Vral A, de Ridder L, Biological dosimetry using the micronucleus assay for lymphocytes : interindervidual differencies in dose response. *Health Phys* 1991 ; 61 : 623~630.
14. Fenech M, Jarvis LR, Morley AA. Preliminary studies on scoring micronuclei by computerized image analysis. *Mutation Res* 1988 ; 203 : 33~38.
15. Tates AD, van Welin MT, Ploem JS. The present state of the automated micronucleus test for lymphocytes. *Int J Radiat Biol* 1990 ; 58 : 813~825.
16. Fenech M, Morley AA. Kinetochore detection in micronuclei : an alternative method for measuring chromosome loss. *Mutagenesis* 1989 ; 4 : 98~104.
17. Aleixander C, Miller DA, Mitchell AR, et al. p82H identifies sequences at every human centromere. *Hum genet* 1987 ; 77 : 46~50.