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A Study on Recovery from Potentially Lethal Damage Induced by γ-Irradiation in Plateau-phase Vero Cells in vitro

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Recovery from potentially lethal damage (PLDR) after irradiation was studied in plateau-phase culture of Vero cells in vitro.

Unfed plateau-phase cells were irradiated wth dose of 1 to 9 Gy using Cs-137 irradiator. Cells then were incubated again and left in situ for 0,1,2,3,4,5,6, and 24 hours and then were trypsinized, explanted, and subcultured in fresh RPMI-1640 media containing 0.33% agar. Cell survival was measured by colony forming ability. An adequate number of heavily irradiated Vero cells were added as feeder cells to make the total cell number constant in every culture dish.

As the postirradiation in situ incubation time increased, surviving fraction increased by PLDR. The rate of PLDR was so rapid that increased surviving fraction reached saturation level at 2 to 4 hours after in situ incubation. As the radiation dose increased, the rate of PLDR fastened and the magnitude of increased surviving fraction at saturation level by PLDR also increased.

In analysis of cell survival curve fitted to the linear-quadratic model, the linear inactivation coefficient (α) decreased largely and reached nearly to zero but the quadratic inactivation coefficient (β) increased minimally by increment of postirradiation in situ incubation time. So PLDR mainly affected the damage expressed as α. In the multitarget model, significant change was not obtained in D_0 but in D_q. Therefore, shoulder region in cell survival curve was mainly affected by PLDR and terminal slope was not influenced at all. And dose-modifying factor by PLDR was relatively higher in shoulder region, that is, in low dose area below 3 Gy.

Key Words: Potentially lethal damage repair (PLDR), Plateau-phase, Linear-quadratic model, Vero cells

INTRODUCTION

Viability of irradiated mammalian cells depend on three categories of damage imposed. Lethal damage is irreversible, irreparable, and leads to cell death; sublethal damage (SLD) can be repaired within hours unless additional SLD is accumulated; and potentially lethal damage (PLD) can lead to cell death if expressed and to cell survival if repaired, influenced by postirradiation environmental condition[19].

Potentially lethal damage repair (PLDR) was known to have similiarity with liquid holding recovery (LHR) that observed among microbes such as *Escherichia coli* B, *E. coli* K-12, and *Saccharomyces cerevisiae*[40]. LHR is an interesting phenomenon that when microbes are left in non-nutrient media or in darkness to delay cell division and then cultured in nutrient media after exposing to ultraviolet or ionizing radiation, more colonies are formed than done when cultured in nutrient media immediatly. It is suggested that damaged DNA are repaired by excision repair mechanism in which certain genes are actively involved during left period after irradiation[4,6,12,14,15].

In mammalian cell lines, PLDR has been confirmed to exist. PLD or PLDR is the most recently discovered radiation damage or repair and their role in radiotherapy for tumor control is recently evaluated as potential importance. After report that plateau-phase cells with stationary cell number owing to nutrient deficiency or confluent cell population could be an good in vitro model for in vivo tumor, researches on PLD or PLDR have been concentrated on plateau-phase cell system. As

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decreased mitosis resulted from rapidly reduced rate of DNA synthesis is in equilibrium with cell death, the total cell number is in static state and most of cells are in G_1 or G_0 phase in the cell cycle[18,28,36].

PLDR was observed in suboptimal growth condition after irradiation and magnitude of PLDR increased in proportion to exposed time in the condition and then reached a saturated level according to many assays using animal cell lines; normal[3,20,27,35,46] or tumor[2,17,32,33] and human cell lines; normal[23~26] or tumor[37,39,41~44]. Furthermore, mechanism of PLDR was suggested to have partial analogy to that of LHR by studies of cell lines deficient of DNA repair in molecular level[22,38,40,45]. But there have been debates on how PLDR affects cell survival curve by postirradation conditional time. PLDR was reported to change slope (D_0) in high dose range only, while recent studies have shown contradictory result that slope is not changed but shoulder region in low dose range is influenced by PLDR[9,16,21,35].

In this experiment, we aimed to clarify kinetic patterns of PLDR and analyze effect of PLDR on cell survival curve after low-dose irradiation using an normal animal cell line in unfed plateau phase in vitro. Postirradiation environmental condition of PLDR was postirradiation in situ incubation.

MATERIALS AND METHODS

1. Cell Line

Vero cells derived from kidney cells of normal adult African green monkey were used. Passage number was 135. The cells were preserved at -70℃ in cryomedia consisted of DMEM (Dulbecco's modified Eagle's media, Flow Lab.) 40%, FBS (Fetal bovine serum, GIBCO) 50%, and DMSO (dimethyl sulfoxide) 10% and used after thawing.

2. Incubation

RPMI 1640 (Flow Lab.) with FBS 5%, Sodium bicarbonate 24 mM/L, HEPES 20 mM/L, and gentamicin 50μg/ml was used as growth media. Cells in T-25 tissue culture flasks (Falcon) were incubated in CO_2 incubator with CO_2 5%, 37℃, full humidification. Vero cells reached plateau phase at day 6 and continued till day 12 after cultur with the initial cell density of 4×10^5 cells/25 cm^2 (Fig. 1). The population doubling time in exponential phase was 22.7 to 30.1 hours and the saturation density at plateau phase was 7.72 ($\pm$0.91)$\times10^6$ cells/25 cm^2 (95% C.I.), that is, 20 times as many as the initial cell density. The plateau phase in the experiment was determined as periods between day 7 and 9 after culture. Cells grew and formed monolayer attached to growth surface, the bottom of tissue culure flasks, and were confluent at plateau phase (Fig. 2).

3. Viable Cell Count

To explant cells attached at growth surface, media were discarded and cells were rinsed 2 times with sterile PBS (phosphate buffered saline, pH 7.2), then were exposed to 0.2 to 0.3 ml solution mixed with 0.05% trypsin (1:250) and 0.02% EDTA for 5 to 10 minutes at room temperature. After inhibiting trypsin action by adding 5 ml RPMI-1640 media, pipetted to make detached cells to single cell suspension (SCS). Dye exclusion method was used to count viable cells in single cell suspension[29]. Mixing SCS with the same amount of 0.2% trypan blue, unstained cells were counted as viable ones using hemocytometer (Improved Neubauer, AO) and phase-contrast microscope (Microstar, AO). Four chamber countings were averaged.

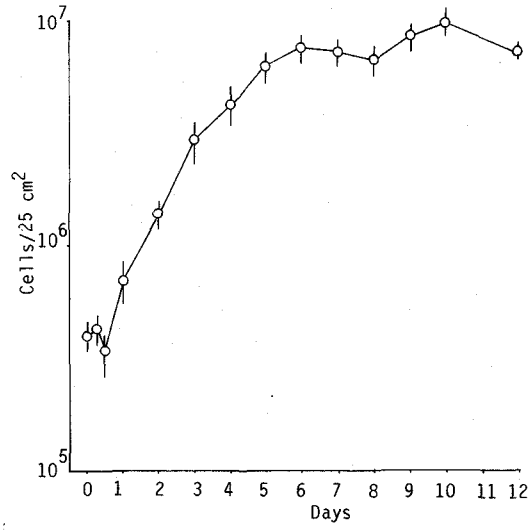

Fig. 1. Growth of Vero cells. 4 x 10^5 cells were plated in replicate T-25 tissue culture flasks, and media were not changed (unfed). Each day cells from two flasks except 0, 6, and 12 hr points were trypsinized and counted by hemocytometer. The plotted points represent average four readings and error bars indicate two standard errors.

4. Irradiation

Cells in air-tight flasks got radiation in the Cs-137 irradiator (Mark Type I, Shepherd) at room temperature. On circular lucite plate set to rotate 6 times a minute, flasks were symmetrically posited to be irradiated evenly. The dose rate was 3.8 Gy/min.

5. Determination of Viability of Irradiated Cells

Cell viability after irradiation was determined by colony forming ability. After irradiation cells were trypsinized, explanted, and subcultured in fresh RPMI-1640 media containing 0.33% Agarose type III at low density. And as the plating efficiency (P.E.) depends on cell density,[30] a variable number of heavily (16 Gy) irradiated plateau phase Vero cells were added as feeder cells to the cell population to be tested for clonogencity so that the cell density in all dishes with agar media might be constant (5×10^5 cells/dish).

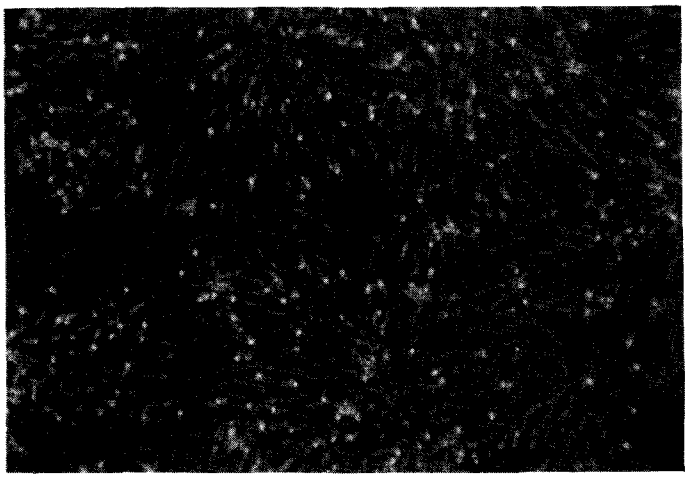

Fig. 2. Morphology of Vero cells in plateau phase. Anchorage dependent cells like fibroblasts were confluent in T-25 flask.

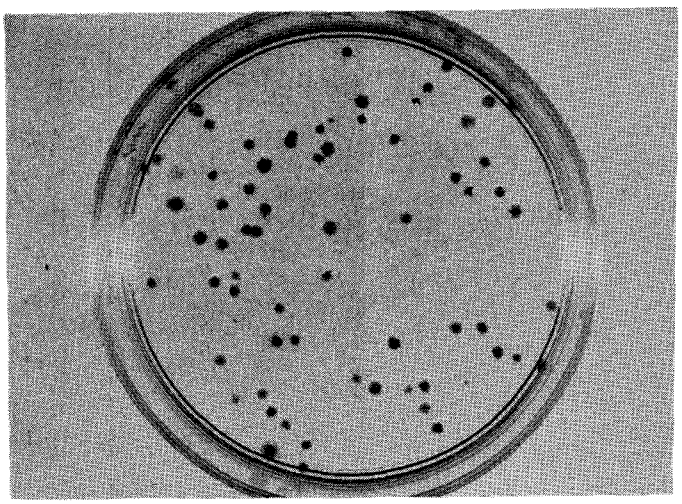

Fig. 3. Morphology of colonies formed in RPMI-1640 media containing 0.33% Agarose on 12 days after culture. Colonies were stained with 0.5% crystal violet, round and well demarcated.

We modified the Freshney's semisolid culture method[13] for colony formation. RPMI-1640 media containing 50 v/v% of 0.66% Agarose Type III (Sigma, A-6138) were kept in fluid state at 42℃. Irradiated SCS were mixed with feeder SCS in ice bath after dilution needed and make the mixed SCS to be 2.5 ml in tube, by adding culture media without agar if necessary. And 2.5 ml media containing 0.66% agar were added to this mixed SCS, stirred to be mixed evenly, and placed into tissue culture dishes (Falcon, 60 mm). Before semisolidifying completely, dishes were overlayed with 0.5 ml media containing 0.66% agar and then subcultured.

Stained with 0.5% crystal violet (Sigma) in 11 to 13 days after subculture, colonies consisted of more than 50 cells were considered as clonal proliferation from a viable cell after irradiation and counted. Size of the viable colonies were larger than 0.5 mm in diameter. Microscope and naked eyes were used in counting colony. Colonies were formed at the bottom of dishes, shaped round with distinct margin easy to count. (Fig. 3)

6. Experimental Design

Cryo-preserved cells were thawed and incubated with initial cell density of 4×10^5 to 5×10^5 cells/25 cm^2. In plateau phase, cells were irradiated with dose of 0,1,3,6 and 9 Gy, and then were placed immediatly again in incubator and left in situ without changing media for 0,1,2,3,4,5,6, and 24 hours. At each time, 1 flask were removed from incubator and cells in the flask were trypsinized, explanted, and subcultured in 4 replicate dishes. Number of irradiated cells in each dish were determined to form 50 to 200 colonies by PE of pilot assay. And the total number of cells in dishes were nearly constant, from 5.00×10^5 to 5.10×10^5. (Table 1,2)

7. Analysis

Surviving fraction (SF) after irradiation was calculated by ratio of PE of irradiated cells to PE of control cells;

$$SF=\frac{\text{PE of irradiated cells}}{\text{PE of control cells}}$$

$$PE=\frac{\text{No. of viable colony}}{\text{No. of cultured cell*}}$$

Table 1. Experimental Grouping for PLD Repair

Radiation dose (Gy)	Number of culture dishes at time (hour) of incubation in situ after iradiation							
	0	1	2	3	4	5	6	24
0	4							4
1	4	4	4	4	4	4	4	4
3	4	4	4	4	4	4	4	4
6	4	4	4	4	4	4	4	4
9	4	4	4	4	4	4	4	4

Table 2. Mixture Conditions of Inoculation to Tissue Culture Dishes with Fresh Semi-Soid Media

Radiation Dose (Gy)	Numbers of cells		No's of dishes	Total numbers of cells in dishes containing fresh agarose media
	Viable	Feeder*		
0	10^3	5×10^5	2	5.01×10^5
	5×10^3	5×10^5	2	5.05×10^5
1	10^3	5×10^5	2	5.01×10^5
	5×10^3	5×10^5	2	5.05×10^5
3	5×10^3	5×10^5	2	5.05×10^5
	10^4	5×10^5	2	5.10×10^5
6	5×10^4	4.5×10^5	4	5.00×10^5
9	5×10^5	0	4	5.00×10^5

Note * : Vero cells irradiated 16 Gy

(*; excluding feeder cells added)
PE of control cells were 0.55% (0.35 to 0.75: 95% CI) and not fluctuated in plateau phase period in the experiment (Day 7,8, and 9). The ratio of SF at time t (SFt) to SF at time 0 (SFo) was designated as surviving fraction ratio (SFR) by the time left in situ with equal radiation dose.

For the analysis of 'dose-SF' relationship, obtained data were fitted and regressed according to 2 models, linear-quadratic (LQ) model and multitarget model[1,5,19]. Parameters of 2 models were determined by nonlinear regression method of Statistical Analysis System.[31]

RESULT

1. Change in Surviving Fraction

As the in situ time interval between irradiation and subculture (post-irradiation incubation time) increased, SF of Vero cells also increased (Fig. 1).

After irradiation of 1 Gy, SF was steady till 3 hr but abrupt increase of SF was noted at 4 hr and further increase was not observed up to 24 hr. Thus maximum potential of increase of SF was reached and already saturated at 4 hr and its average magnitude was 1.67 in SFR. In 3 Gy, increase in SF appeared to be saturated at 2 hr and even had lowering tendency at 6 and 24 hr. Average level of saturation was 3.32 in SFR. After 6 Gy, the pattern was peculiar and so there was no increase in SF until 24 hr with 9.57 SFR. With 9 Gy, SF increased at 2 hr and its magnitude continued up to 24 hr. Average SFR in saturated SF's was 12.56. Average SFR's in saturated level were all significant compared to SF at time 0 except that observed in 1 Gy.

As the radiation dose increased, the in situ time needed to reach sturation level firstly (Tmax) decreased and the in situ time for 50% of saturation level ($T^1/_2$) also decreased; $T^1/_2$ in 1,3 and 9 Gy were 2, 1, and 1 hr, respectively. Although average SFR in saturated level increased as the radiation dose increased, SFR at 24 hr also had a tendency to be saturated as radiation dose increased; SFR at 24 hr were 1.61, 2.14, 9.57, and 9.25 in 1, 3, 6 and 9 Gy, respectively.

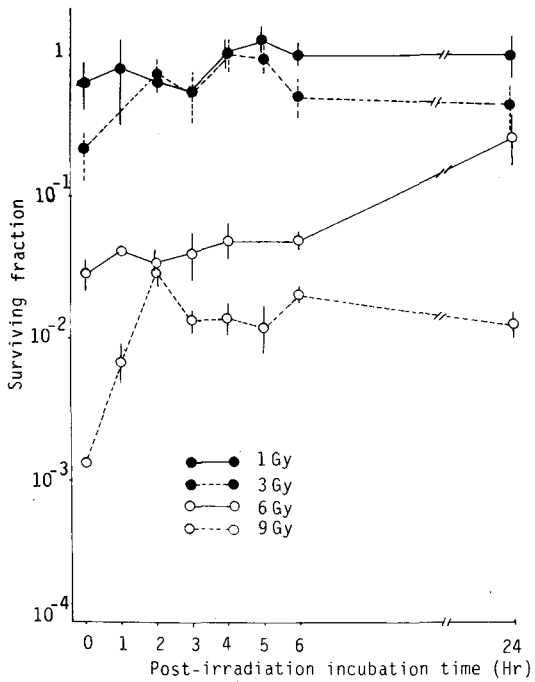

Fig. 4. Survival of unfed plateau phase Vero cells as a function of in situ time to subculture after various doses of Cs-137 gamma-rays. The plotted points represent average of surviving fractions obtained from 4 dishes and bars indicate S.E.

2. Dose-Surviving Fraction Analysis

Parameters by LQ model were tabulated in Table 3. As the postirradiation in situ incubation time increased, linear inactivation coefficient α decreased rapidly and nearly reached to 0 but quadratic inactivation coefficient β slightly increased. Negative α value at 24 hr curve was due to SF above 1 observed in 1 Gy experiment. And this value was adjusted by limiting to zero according to the equation of Fertil & Malaise[11] and then β parameter was corrected. The value of α/β decreased from 11.34 in immediate subculture to 0.53 and 0 (adjusted value of -0.43) in 6 and 24 hr delayed subcultures, respectiviely, as post-

Table 3. Survival Parameters by L–Q Model

Post-irradiation in situ time (hr)	α, Gy^{-1} (95% C.I.)	β, Gy^{-2} (95% C. I.)
0	0.4048 ± 0.0804	0.0357 ± 0.0142
6	0.0298 ± 0.6025	0.0555 ± 0.1067
24	−0.0236 ± 0.4600	0.0545 ± 0.0818
	0*	0.0517*

Note, * ; adjusted values of 24hr in situ time parameters by Fertil & Malaise's equation

irradiation in situ time increased. In linear regression equation for LQ model, there was a conspicuous change of α. (Fig. 5) In cell-survival curves by LQ model, widening of shoulder region in low dose range was major curvature change by increasing postirradiation in situ incubation time while negligible effect could be obtained in high dose range. (Fig. 6)

SFR in LQ model dependend upon radiation dose. In 6 hr in situ time, SFR at 1,3,6, and 9 Gy were 1.43, 2.58, 4.65, and 5.88, respectively. And in 24 hr in situ time, SFR at 1,3,6 and 9 Gy were 1.51, 3.05, 6. 64 and 10.31, respectively. Therefore, SFR increased as to radiation dose upto 9 Gy with sigmoid pattern in both conditions. (Fig. 7)

Dose modifying factor (DMF), ratio of dose needed for same SF in immediate subculture to dose needed in delayed subcultures, exponentially

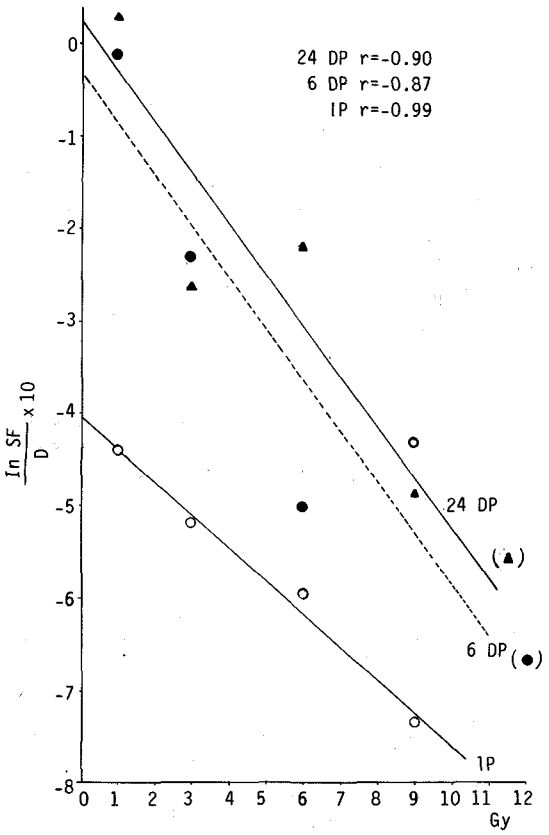

Fig. 5. Inactivation data of Vero cells plotted as (ln SF/D) versus radiation dose D in Gy. SF = surviving fraction. DP means delayed plating ; IP, immediate plating.

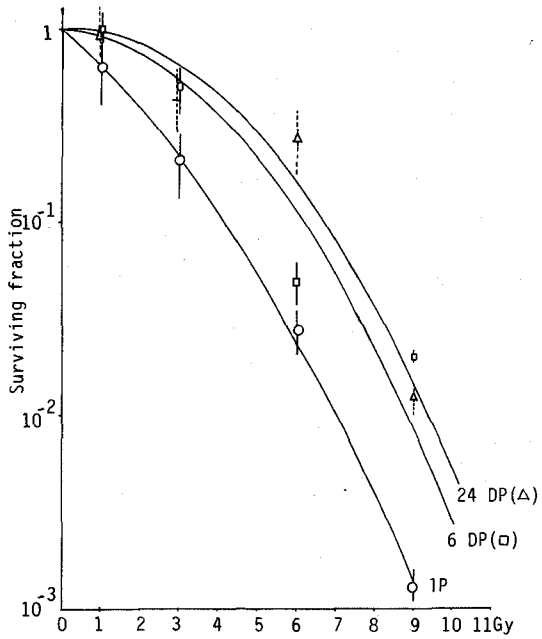

Fig. 6. Single-dose cell survival curves of plateau phase Vero cells by the linear quadratic model. The plotted points represent average of surviving fractions obtained from 4 dishes and bars indicate S.E. DP means delayed plating ; IP, immediate plating.

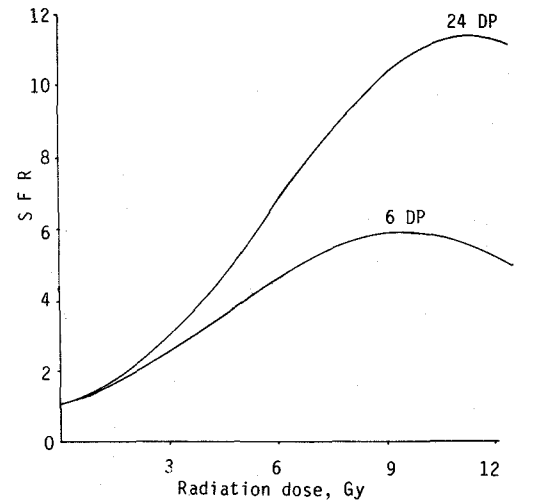

Fig. 7. Surviving fraction ratio (SFR) as a function of radiation dose by the linear quadratic model. DP means delayed plating.

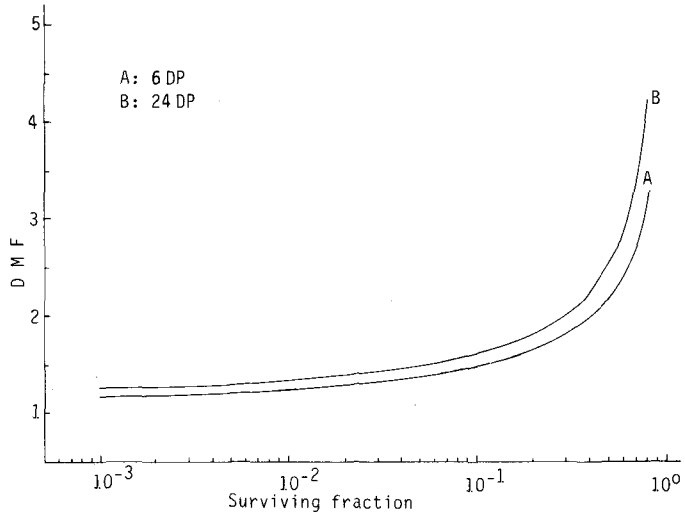

Fig. 8. Dose modifying factors in delayed platings compared with immediate plating by the linearquadratic model. DP means delayed plating.

Table 4. Survival Parameters by Multitarget Model

Post-irradiation in situ time (hr)	n (95% C.I.)	Do, Gy (95% C.I.)	Dq*, Gy (95% C. I.)
0	1.31 ± 0.20	1.65 ± 0.20	0.45 ± 0.27
6	8.50 ± 8.12	1.18 ± 0.42	2.53 ± 1.46
24	2.61 ± 8.88	2.23 ± 4.45	2.14 ± 8.74

Note, Dq : quasithreshold

decreased as SF decreasd in log scale, that is, DMF in low dose range below 3 Gy was 3 or 4 times as high as DMF in high dose range if subculture time was equal. (Fig. 8)

Among the parameters by multitarget model, significant change was noted in Dq, especially between immediate subculture and 6 hr delayed subculture, meanwhile other parameters, Do and n, were not affected greatly by the post-irradiation in situ incubation time. This also meaned that increase in SF by delayed subculture after irradiation mainly resulted in widening of the initial shoulder region at cell survival curve. (Table 4)

DISCUSSION

Increase in viable cell number by selective detachment of dead cells from flask or cell proliferation did not cause SF of Vero cels to increase after postirradiation incubation in situ. Because there was no increase in numbers of cells that were detached and floating freely in the media till 24 hours' incubation and, what is more important, irradiated cells were in the plateau phase when cell number was stationary and environmental factors inducing cellular proliferation were not applied to the plateau-phase cells. Therefore this must be caused by repair of damaged cells during postirradiation incubation.

Assuming that Nt is the number of viable colonies formed from Vero cells which were incubated again in situ for t hours after irradiation and then were subcultured, Nt can be expressed as following;

$$Nt = N + SLDRT + PLDRt$$

where

N=colony No. from cells without damage

SLDRt=colony No. from cells repaired from SLD
PLDRt=colony No. from cells repaired from PLD
t=in situ incubation time, hours

So difference between Nt and No (immediate subculture) is;

$$Nt - No = (SLDRt - SLDRo) + (PLDRt - PLDRo)$$

Because cells had single irradation and cell density was constant at the time of irradiation, SLD amount was equal and SLDR was also equal without regard to in situ time,[10] thus SLDRt = SLDRo. And at t=0, all cells with PLD had to be dead and did not form a colony at all, and consequently PLDRo=0 because immediate subculture after irradiation in fresh nturient media provided Vero cells with optimal growth condition enough to express PLD. Therefore above equation can be shortened as

$$Nt - No = PLDRt \text{ or } Nt = No + PLDRt \quad (No = N)$$

Consequently, increase in SF, observed in cells incubated in situ for t hours immediatly after irradiation and before subculture, was due to the additionally formed colonies from cells recovered from PLD during that period, otherwise be lethal by PLD expression.

When feeder cells, heavily irradiated plateau-phase Vero cells, only were cultured, $N_0=0$ and $N_{24}=2\pm1$ (95% CI). So had the feeder cells in the experiment PLDR potential. Because feeder cells were added to the cells to be tested for colony forming ability just before subculture in fresh nutrient media in low cell density, feeder cells and their PLDR ability did not affect and confound Vero cells' recovery from PLD and ability of colony formation.

In general, surviving fraction increased rapidly and reached saturated level by PLDR when cells were irradiated in vitro in plateau phase and left in situ. Kinetic analyses of PLDR showed a range of Tmax from 2~4 hours[37] to 4~6 hours[16,17,25,45] and that of $T^1/_2$ 1~2 hours[16,25]. In a report[46], PLDR was observed to start 3 minutes after postirradiation incubation. Thus the rate of PLDR seems to be fast irrespective of cells types. Another important aspect of PLDR is that the magnitude of PLDR on saturated level is dependent on radiation dose. In this experiment, saturated magnitude showed dose dependent pattern with saturating tendency. Dose threshold for PLDR suggested by a report that PLD was not repaired below 7.5 Gy[32] is not confirmed in this study.

The more radiation dose to Vero cells, the faster the rate of PLDR and the shorter T_{max} or $T^1/_2$. These results are consistent with a report that the higher the relative pyrimidine dimer load on the DNA of fibroblasts by ultraviolet irradiation, the faster the rate of its removal, suggesting that the overall reaction is dependent on the concentration of the substrate[22].

Surviving fraction ratio at Tmax with 5 to 10 Gy ranged 1.5 to 30 in other cell lines[7,44], and so that ranged 10 to 13 in this study is of moderate magnitude. Surviving fraction around 1, which meaned complete repair of PLD, with 1 and 3 Gy indicates that reparability of Vero cells has high potential enough to overcome damages induced by low dose radiation.

Cotroversy about effect of PLDR on cell survival curve still remains. Traditional belief was that PLDR decreased the terminal slope of the multitarget model (Do: mean lethal dose)[12,25,32,37,45,46]. To the contrary, recent analyses have shown that PLDR mainly increased shoulder region without changing Do[9,16,21,33,35]. And some of recent workers even insists on 'in situ repair' and 'contact effect' as indepdent components of PLDR. In this study, Do was not affected but Dq was significantly increased, and α decreased greatly but β increased minimally, a result indicates that single-hit damage expressed as α is the site of PLDR. In LQ model, double-strand breaks of DNA is expressed as α, parameter of cell killing by single-hit mechanism, and is more irreparable than single-strand breaks of DNA which expressed as β, parameter of cell killing by double-hit mechanism. But it was recently reported that a large component of the linear mechanism of cell inactivation could be protected by scavenging the indirect action of OH radicals[5]. PLDR decreased both, α and β in a report[7] and evidence consistent with this experiment was published that a fibroblast deficient of glutathione synthetase which is involved in DNA repair and scavenging free radicals induced by irradiation had the higher value of α than and about the same vaue of β as a normal fibroblast had[8]. In these respect, it might be said that the main type of damage affected by PLDR after low-LET radiation is the damage expressed as α parameter in LQ model.

When SFR was extrpolated, it decreased after maximum value (Fig. 7). This suggests that after certain dose limit the reparability of PLD might decrease or the amount of reparable PLD itself might decrease. And higher DMF observed in low dose region (Fig. 8) strongly reflects appearing of shoulder region with great curvature by PLDR.

Although we cannot apply the results obtained

by in vitro assay directly to in vivo system, it has a particular meaning that cell lines derived from osteosarcoma or malignant melanoma, clinically radioresistant, showed ever-increasing patterns of PLDR after conventional cell lines reached saturating level of PLDR[39,43], and that PLDR has been observed not only after delivery of ionizing radiation or ultraviolet but also treatment of antimetabolites or cytotoxic agents[34]. Molecular or genetic investigation of debated and unsettled nature and patterns of potentially lethal damage repair will lead to understanding of cellular repair mechanism in detail, modification of conventional treatment against malignant tumor, and development of controlling method of PLDR itself for tumor control.

REFERENCES

1. **Alper T:** Survival curve models. 'In' Radiation Biology in Cancer Research. Meyn RE, Withers HR (eds), Raven Press, New York, 3-18, 1980
2. **Beer JZ, Lett JT, Alexander P:** Influence of temperature and medium on X-ray sensitivities of leukemia cells in vitro. Nature 199:193-194, 1963
3. **Belli JA, Shelton M:** Potentially lethal radiation damage: repair by mammalian cells in culture. Science 165:490-492, 1969
4. **Castellani A, Jagger J, Setlow RB:** Overlap of photoreactivation and liquid holding recovery in Escherichia coli B. Science 143:1170-1171, 1964
5. **Chapman JD:** Biophysical models of mammalian cell inactivation by radiation. 'In' Radiation Biology in Cancer Research. Meyn RE, Withers HR (ed), Raven Press, New York, 21-32, 1980
6. **Charles RL, Zimmerman LN:** Dark reactivation in ultraviolet irradiated Escherichia coli. J Bacteriol 71:611-616, 1956
7. **Deschavanne PJ, Fertil B, Malaise EP:** Radiosensitivity and repair of radiation damage in human HF19 fibroblasts. Int J Radiat Biol 38:167-177, 1980
8. **Deschavanne PJ, Malaise EP:** Radiation survival of glutathine-deficient human fibroblasts in cultures. Br J Radiol 54:361-362, 1981
9. **Durand RE:** Repair during multifraction exposures: sheroids versus monolayers. Br J Cancer 49, Suppl. VI: 203-236, 1984
10. **Durand RE, Sutherland RM:** Effects of intercellular contact on repair of radiation damage. Exp Cell Res 71:75-80, 1972
11. **Fertil B, Malaise EP:** Inherent celluar radiosenstivity as a basic concept for human tumor radiotherapy. Int J Radiat Oncol Biol Phys 7:621-629, 1981
12. **Frankenberg D, Frankenberg-Schwager M:** Interpretation of the shoulder of dose-response curves with immediate plating in terms of repair of potentially lethal lesions during a restricted time period. Int J Radiat Biol 39:617-631, 1981
13. **Freshney RI:** Culture of Animal Cells. A Manual of Basic Technique. Alan R Liss, Inc, New York, 133-136, 1983
14. **Ganesan AK, Smith KC:** Dark recovery processes in Escherichia coli irradiated with ultraviolet light. I. effect of rec⁻ mutations on liquid holding recovery. J Bacteriol 96:365-373, 1968
15. **Ganesan AK, Smith KC:** Dark recovery processes in Escherichia coli irradiated with ultraviolet light. II. effect of uvr genes on liquid holding recovery. J Bacteriol 97:1129-1133, 1969
16. **Gould MN, Cathers LE, Clifton KH, et al:** The influence of in situ repair systems on survival of several irradiated parenchymal cell types. Br J Cancer 49, Suppl VI:191-195, 1984
17. **Hahn GM, Rockwell S, Kallman RF, et al:** Repair of potentially lethal damage in vivo in solid tumor cells after x-irradiation. Cancer Res 34:351-354, 1974
18. **Hahn GM, Stewart JR, Yang SJ, et al:** Chinese hamster cell monolayer cultures. I. changes in cell dynamics and modifications of the cell cycle with the period of growth. Exp Cell Res 49:285-292, 1968
19. **Hall EJ:** Radiobiology for the radiologists. 2nd ed, Harper & Row, Philadelphia 129-170, 1978
20. **Hall EJ, Kraljevic U:** Repair of potentially lethal radiation damage: comparison of neutron and x-ray RBE and implications for radiation therapy. Radiology 121:731-735, 1978
21. **Jirtle RL, Michalopoulos G, Strom SC, et al:** The survival of parenchymal hepatocytes irradiated with low and high LET radiation. Br J Cancer 49, Suppl VI: 197-201, 1984
22. **Konze-Thomas B, Levinson JW, Maher VM, et al:** Correlation among the rates of dimer excision, DNA repair replication, and recovery of human cells from potentially lethal damage induced by ultraviolet radiation. Biophy J 28:315-325, 1979
23. **Little JB:** Repair of sublethal and potentially lethal radiation damage in plateau phase cultures of human cells. Nature 224:804-806, 1969
24. **Little JB:** Differential response of rapidly and slowly proliferating human cells to X irradiation. Radiology 93:307-313, 1969
25. **Little JB, Hahn GM:** Life-cycle dependence of repair of potentially-lethal radiation damage. Int J Radiat Biol 23:401-407, 1973
26. **Little JB, Hahn GM, Frindel E, et al:** Repair of potentially lethal radiation damage in vitro and in vivo. Radiology 106:689-694, 1973
27. **Mauro F, Elkind MM:** Sulfur mustard and X-ray: difference in expression of lethal damage. Science

155:1561-1563, 1967

28. **Mauro F, Falpo B, Briganti G, et al:** Effects of antineoplastic drugs on plateau-phase culutres of mammalian cells. I. description of the plateau-phase system. J Nat Cancer Inst 52:705-713, 1974
29. **Patterson Jr MK:** Measurement of growth and viability of cells in cultures. Methods Enzymol 58: 141-152, 1979
30. **Révész L:** Effect of lethally damaged tumor cells upon the development of admixed viable cells. J Nat Cancer Inst 20:1157-1186, 1958
31. **SAS Institute:** SAS user's guide. statistics. SAS Inst Inc, Cary, North Carolina, 15-38, 1982
32. **Shibamoto Y, Takahashi M, Ono K, et al:** Recovery from potentially lethal damage after X-irradiation in three experimental tumors in mice. Jpn J Cancer Res 76:278-283, 1985
33. **Shipley WU, Stanley JA, Courtenay VD, et al:** Repair of radiation damage in Lewis lung carcinoma cells following in situ treatment with fast neutrons and gamma-rays. Cancer Res 35:932-938, 1975
34. **Twentyman PR:** Timings of assay: An important consideration in the determination of clongenic cell survival both in vitro and in vivo. Int J Radiat Oncol Biol Phys 5:1213-1220, 1979
35. **Utsumi H, Elkind MM:** Two forms of potentially lethal damage have similar repair kinetics in plateau-and log-phase cells. Int J Radiat Biol 47: 569-580, 1985
36. **Watanabe I, Okada S:** Stationary phase of cultured mammalian cells (L5178Y). J Cell Biol 35:285-294, 1967
37. **Weichelbaum RR, Little JB, Nove J:** Response of human osteosarcoma in vitro to irradiation: evidence for unusual cellular repair capacity. Int J Radiat Biol 31:295-299, 1977
38. **Weichelbaum RR, Nove J, Little JB:** Deficient recovery from potentially lethal radiation damage in ataxia telangiectasia and xeroderma pigmentosum. Nature 271:261-262, 1978
39. **Weichelbaum RR, Nove J, Little JB:** X-ray sensitivity of human tumor cells in vitro. Int J Radiat Oncol Biol Phys 6:437-440, 1980
40. **Weichelbaum RR, Little JB:** Repair of potentially lethal X-ray damage and possible applications to clinical radiotherapy. Int J Radiat Oncol Biol Phys 9:91-96, 1983
41. **Weichelbaum RR, Little JB:** The differential response of human tumors to fractionated radiation may be due to a post-irradiation repair process. Br J Cancer 46:532-537, 1982
42. **Weichelbaum RR, Little JB:** Radioresistance in some human tumor cells conferred in vitro by repair of potentially lethal x-ray damage. Radiology 145:511-513, 1982
43. **Weichelbaum RR, Malcolm AW, Little JB:** Fraction size and the repair of potentially lethal radiation damage in a human melanoma cell line. Radiology 142:225-227, 1982
44. **Weichelbaum RR, Schmit A, Little JB:** Cellular repair factors influencing radiocurability of human malignant tumors. Br J Cancer 45:10-16, 1982
45. **Weichelbaum RR, Tomkinson K, Little JB:** Repair of potentially lethal damage in fibroblasts derived from patients with hereditary and D-deletion retinoblastoma. Int J Radit Biol 47:445-447, 1985
46. **Winans LF, Dewey WC, Dettor CM;** Repair of sublethal and potentially lethal X-ray damage in synchronous Chinese hamster cells. Radiat Res 52: 333-351, 1972

국문초록 =

평형기의 Vero 세포계에서 방사선(γ-선) 조사후 발생한 잠재치사 손상의 회복에 관한 연구

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방사선 조사후 발생한 잠재치사손상의 회복(PLDR)에 있어 조사선량 및 시간에 따른 환경변화가 회복의 동적양상에 미치는 영향을 Vero 세포계를 이용하여 실험하였다.

배양액을 교환시키지 않고 배양하여 평형기에 도달한 세포에 동물실험용 세시움 조사기로 1 Gy~9 Gy의 감마선을 조사하고 각 조사조건에서 0~6 및 24 시간동안 정치시킨 후 Agarose가 포함된 새로운 배양액에서 배양하였다. 16 Gy를 조사한 동종의 세포를 feeder 세포로 첨가하여 배양액내의 전체세포수를 일정하게 한 조건에서 형성된 세포집락수에 따라 세포의 생존을 정하였다.

잠재치사손상의 회복은 2~4시간 정치후에 포화수준에 도달한 빠른 회복이었다. 방사선량이 증가함에 따라 회복속도는 증가하였고, 포화수준의 회복량도 증가하였다.

Linear-quadratic model에 의한 '방사선량-생존분획' 분석결과 잠재치사손상이 회복됨에 따라 일차 비활성계수 α는 급속히 감소하여 0에 접근하였고 이차 비활성계수 β는 미미하게 증가하여 PLDR은 α로 표시되는 손상에 주로 영향을 주었다. Multitarget model에 따라 분석한 결과 Do는 변화가 없고 Dq가 증가하였다.

세포 생존분획이 높은 3 Gy 이하의 저선량 영역에서 dose modifying factor가 높아 잠재치사손상의 회복에 의한 영향이 저선량 영역에서 상대적으로 크게 나타났다.

중심단어; 잠재치사손상의 회복-방사선 조사-평형기 Vero 세포 세포