REPAIR OF SINGLE DNA-STRAND BREAKS AND RECOVERY FROM SUBLETHAL RADIATION INJURY

YOSHIDA, Mineo

(Citation)
The Kobe journal of the medical sciences,24(1):31-47

(Issue Date)
1978-03

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100488899

Kobe J. Med. Sci. 24, 31 - 47, March 1978

REPAIR OF SINGLE DNA-STRAND BREAKS AND RECOVERY FROM SUBLETHAL RADIATION INJURY*

Mineo YOSHIDA

Department of Radiation Biophysics
Kobe University School of Medicine

INDEXING WORDS

single-strand break repair; recovery from sublethal injury; cytosine arabinoside; repair replication; X-rays; dose fractionation

SYNOPSIS

I investigated the effects of cytosine arabinoside (araC) on single-strand break (SSB) repair, repair replication and recovery of sublethal damage (SLD) in X-irradiated HeLa S3 cells. The most effective araC treatment was the protocol involving addition and presence of araC (10^{-5} M) before (30 minutes) and for various lengths of time after X-irradiation of the cells. AraC partially inhibited SSB repair and repair replication after 20 krad, and almost completely SLD recovery after relatively low X-ray doses, strongly suggesting a new finding that SSB repair requiring repair replication at the molecular level may be at least in part responsible for SLD recovery at the cellular level. It seems that this result will shed further light on the combined chemoradiotherapy of cancers, especially using low LET radiation.

---

*This is the thesis for Doctor Degree of Medical Sciences.

The abbreviations used: araC, cytosine arabinoside; TdR, thymidine; FUdR, 5-fluoro-2'-deoxyuridine; BUdR, 5-bromo-2'-deoxyuridine; CdR, deoxycytosine; HU, hydroxyurea; RNase, ribonuclease; SSB, single strand break; SLD, sublethal damage; LET, linear energy transfer.

Received for publication June 14, 1977

Author's name in Japanese: 吉田峯雄

INTRODUCTION

Cellular and molecular mechanisms by which cells resist the radiation killing are extremely important for radiotherapy of cancers.[1,21] Recoveries from SLD[3,4,5,18,19,20] and from potentially lethal damage[13] by relatively low doses of low LET radiation have been well studied in a wide variety of mammalian cell lines derived from either normal or malignant origins.[21] The Elkind model of SLD recovery involves the initial cell repair (recovery) process giving rise to a maximum survival between 2 to 4 hours after the first irradiation, followed by cell progression or repopulation.[5,18,19]

Extensive attempts were made to elucidate the molecular mechanism of SLD recovery by inactivating the initial SLD repair by various metabolic inhibitors of DNA, RNA and protein synthesis.[6,19,20] However, almost all have been unfruitful except a few successes that DNA binding agents such as actinomycin D,[6] phleomycin [20] and proflavine (Fujiwara, unpublished data) and a long-term pretreatment with cycloheximide (a protein synthesis inhibitor)[20] suppress SLD recovery in X-irradiated Chinese hamster and mouse L cells. That low doses of actinomycin D are effective in inhibiting both SLD recovery[6] and repair of X-ray induced dissociation of a DNA complex[2] is only one correlation ever reported. On the other hand, more than 90% of SSB's are repaired rapidly within 60 minutes (see Painter's review,[15] ref. 9) and SSB repair is not inhibited by low doses of actinomycin D.[2] Thus, it is implied that the time-dose and molecular relationships between SSB repair and SLD recovery are not always correlated. Further, Sato et al.[17] have proposed that radiation induced cell-surface damage is related with sublethal injury. At present the situation is still conflicting.

My attempts here are to study the effects of araC on SSB repair and repair replication, and to correlate these results with araC effect on SLD recovery in X-irradiated HeLa S3 cells.

MATERIALS AND METHODS

Cells and Culture.

The cell line used was HeLa S3-91V, maintained for a long

time in our laboratory.[7,10] The cells were cultured in TdR-free Ham's F10HI medium[14] supplemented with 10% calf serum (Flow Lab.) under a water-saturated atmosphere of 5% CO_2-95% air, as described previously by Fujiwara et al.[7,10]

X-ray Irradiation.

A Toshiba Model KXC 18-2 therapeutic unit was used by operating at 190 kV and 25 mA with a 1.5 mm Al + 0.5 mm Cu filter for cell survival and with a built-in filter of 0.5 mm Al for SSB. The dose rates, measured by the Fricke's ferrous sulfate method and Victreen condenser R-meter (Victreen Co.), were 83 rad/min. and 1.35 krad/min. for survival and SSB studies, respectively.[9] Irradiation of the cells with 0 or 20 krad to produce SSB's was performed at or near 0°C to suppress rejoining during irradiation.[9]

Survival Assay.

HeLa S3 cells in log-growth phase were trypsinized and plated in appropriate numbers in duplicate Falcon plastic Petri dishes (60 mm x 15 mm, Division of BioQuest). After preincubation for 4 to 6 hours at 37°C, the cells were exposed to X-rays and/or chemicals according to the following protocols: (1) Ordinal survival curve after serial single doses (0 to 1000 rad); (2) fractionated dose survival curve after the first fixed dose (X_1 = 240 rad, which exceeds the shoulder region), followed by the second serial doses (x_2) at the 2 hours' interval (X_1-2 hours-x_2); (3) fractionated dose survival kinetics after equal split doses ($X_1 = x_2$ = 240 rad) at various time intervals (240 rad-t-240 rad). In the protocols,[2,3] 10^{-5} M araC was sometimes added 30 minutes before and/or for various lengths of time after the first dose (see Figs. 5 and 6), to see whether or not araC affects SLD repair. After araC treatments, 5 x 10^{-4} M CdR was added to the plates to reverse the araC inhibition and hence to allow colony formation. Then, the plates were incubated for 12 to 14 days until visible colonies with 50 cells or more developed.

Radioactive Labelling of Cells.

(a) For DNA sedimentation in alkaline sucrose gradients (see below), 10^5 cells in log phase were labelled for 24 to 48 hours with 0.1 to 0.5 μCi/ml of [^{3}H-methyl]TdR (5 Ci/mmole, Radiochemical Centre). Radioactive medium was removed and replaced with normal medium for several hours prior to X-irradiation. (b) For repair replication, 10^7 cells pretreated with 10^{-6} M FUdR for 2

hours and with 2 x 10^{-3} M HU for the last 30 minutes were irradiated with 0 or 20 krad at 0°C, and further incubated for 4 hours at 37°C in 10^{-5} M [6-^{3}H]BUdR (10 μCi/ml, sp. act., 523 mCi/mmole, Radiochemical Centre), 10^{-6} M FUdR and 2 x 10^{-3} M HU.[16]

DNA Isolation and CsCl Isopycnic Centrifugation.

The DNA from the cells labelled according to *protocol (b)* was isolated by a modification of Marmur's method, as described previously.[10] DNA preparation was further treated with 25 μg/ml of preheated pancreatic RNase (Worthington) and 50 units/ml of T1 RNase (Sankyo). The initial density of a neutral CsCl solution in 0.1 M saline-0.01 M citrate containing 50 μg/ml of the DNA was adjusted to 1.72 g/ml. The density equilibrium was attained by centrifugation at 33000 rpm for 48 hours at 20°C in an SW50.1 rotor of a Beckman Model L5-50 ultracentrifuge (Spinco Division), as described previously.[11] After the run, 6-drop fractions were collected from the bottoms of the tubes, and densities of the individual fractions were measured by refractometry at 25°C.[11] Repair incorporation of [^{3}H]BUdR is known to be restricted to the light DNA band at 1.700 g/ml.[16] However, the first neutral CsCl light band contained a fraction of residually replicated DNA in the presence of HU. In order to remove this fraction and to reveal non-semiconservative repair synthesis, DNA around the buoyant density of 1.700 g/ml in neutral CsCl was pooled and dialysed. The same amount of DNA (33 μg/tube) was rebanded in alkaline CsCl (initial density = 1.76 g/ml in 0.1 M Na_2HPO_4, 0.1 M NaOH and 10^{-3} M EDTA).

Alkaline Sucrose Sedimentation.

The entire method was described elsewhere.[7,8,9,10,11] The following is a brief description (see Fig. 1). The cells labelled according to *protocol (a)* were lysed, and the finally alkalinized lysate, constituting about 5 - 10 x 10^{3} lysed cells/0.2-ml loading volume, was layered on top of 4.8 ml of 5 to 20% (w/v) linear sucrose gradient at pH 12.5. The loaded tubes were centrifuged at 35000 rpm for 1 hour at 20°C in an SW50.1 rotor. After the run, 25 fractions were usually collected from the bottoms of the tubes, and subjected to assay for radioactivity.

Weight (M_w) and number (M_n) average molecular weights of the DNA were calculated from the sedimentation profiles (fractions 2 to 24), as described previously.[9] The number of SSB's per 1 g

of DNA, N, was calculated by the formula: $N = 6.023 \times 10^{23}(1/Mnt - 1/Mno)$, where Mno and Mnt represent the Mn values of unirradiated and irradiated DNA.[7] The fractions of SSB's remaining at time t after irradiation, $f(t)$, were obtained by the equation: $f(t) = Nt/No$, where No and Nt are the N values at repair times 0 and t, respectively. The $f(t)$ curve vs repair time (t) reveals the kinetics of SSB repair (see Fig. 3).

Measurement of Radioactivity.

Acid-insoluble radioactivity of the individual sucrose and CsCl fractions were collected on paper filter discs and measured in PPO-POPOP-toluene (4 g/0.05 g/1000 ml) using a Packard Model 3330 liquid scintillation spectrometer.[7,8,9.10,11]

RESULTS

Repair Kinetics of X-ray Induced SSB's.

Immediate lysis and centrifugation revealed that alkaline sucrose sedimentation velocity of the DNA of irradiated HeLa S3 cells was much slower than that of unirradiated control (Fig. 1A). Mn of control DNA was about 140×10^6 under the present conditions. A dose of 20 krad produced $\sim 2 \times 10^{16}$ SSB's/g DNA, or $\sim 1 \times 10^{12}$ SSB's/g/rad, as agreed with the value obtained previously in normal human cells.[9] As shown in Figs. 1B (30 minutes) and 1C (60 minutes), the sedimentation velocity shifted towards that of control DNA, indicating that induced SSB's are repaired during post-incubations at 37°C. Fig. 3 (open circle) demonstrates the first-order kinetics of SSB repair up to 60 minutes, a half-life of which is approximately 10 minutes. By 60 minutes of repair time, about 95% of the initial number of SSB's was rejoined. At 120 minutes, about 3% of breaks in alkaline sucrose remained unjoined, presumably showing that this minor fraction appears to result from double strand breaks whose yield is less than 1/10 of the SSB yield. The repair result is essentially the same as reported by Fujiwara et al.[9] in normal human cells and reviewed by Painter.[15]

Effects of AraC on SSB Repair.

Figs. 1D to F show the results after post-X-ray treatments of HeLa S3 cells with 10^{-5} M araC. AraC allowed SSB repair at an almost normal rate until 30 minutes (Fig. 1E), but slowed further

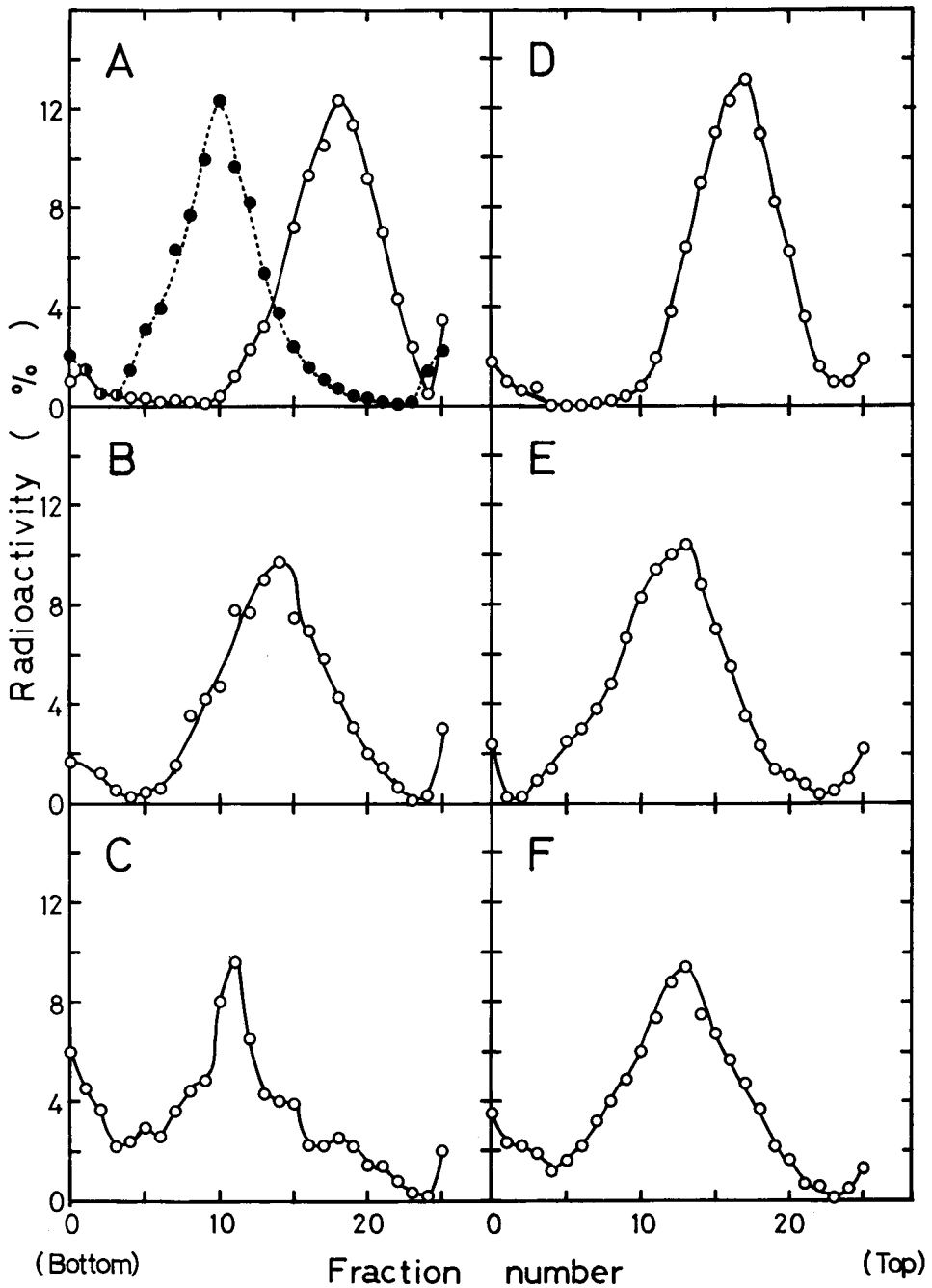
A
B
C
D
E
F
12
8
4
0
Radioactivity (%)
0
10
20
(Bottom)
Fraction number
(Top)

Fig. 1 The production and repair of X-ray induced SSB's (alkaline sucrose profiles). HeLa S3 cells were prelabelled with 0.2 μCi/ml of [^{3}H]TdR for 48 hours, chased, and irradiated with 0 or 20 krad at 0°C, followed by the immediate lysis or further incubations for 30 and 60 minutes at 37°C before lysis. Then, the cells were lysed in 0.25% sodium dodecylsulfate, 0.01 M EDTA and o.15 M Na bicarbonate, pH 8, and digested with 2 mg/ml of preheated Pronase for 4 hours at 37°C. After addition of 0.1 ml of 3M NaOH per 1 ml lysate and adjustment to 5-10 x 10^3 lysed cells/0.2 ml, a 0.2 ml aliquot was layered on top of 4.8 ml of linear alkaline sucrose gradient in 0.8 M NaCl, 0.2 M NaOH, 0.01 M EDTA and 0.015 M *p*-aminosalycilate, pH 12.5. (7,8,9,10,11) The loaded tubes were centrifuged at 35000 rpm for 1 hour at 20°C in an SW50.1 rotor. After the run the gradients were fractionated, and radioactivity was measured. A, lysed immediately after 0 (--●--) or 20 krad (—○—); B, lysed after a 30 minutes' incubation following 20 krad; C, lysed at 60 minutes following 20 krad; D, lysed immediately after 20 krad; E, irradiated, incubated in 10^{-5} M araC for 30 minutes, lysed; F, irradiated, incubated for 60 minutes in 10^{-5} M araC. *Mw* of control DNA was 280 x 10^6.

rejoining as revealed by the similar profiles (peak: fraction 13) between 30 minutes (Fig. 1E) and 60 minutes (Fig. 1F). In order to expect more drastic inhibition of SSB repair, the cells were continuously treated with araC for 30 minutes before irradiation (20 krad) and during repair incubations for 30 and 60 minutes. As expected, the continuous pre- and post-treatments inhibited SSB repair to a greater extent, but not completely (Figs. 2B, 2C). Fig. 3 depicts the kinetics of araC prevention of SSB repair. The presence of araC before and after irradiation [protocol: araC, 30 minutes-X-araC, *t* (=30, 60 minutes)] caused about 2-fold greater inhibition than its absence before irradiation [protocol: X-araC, *t* (=30, 60 minutes)]. Actually, fractions of SSB's remaining *(f(t))* after the former and latter protocols ranged from 60% (30 minutes) to 45% (60 minutes) and from 30% (30 minutes) to 20% (60 minutes), respectively. A higher dose of araC (5 x 10^{-5} M) and a longer pretreatment (60 minutes) did not significantly increase the extent of repair inhibition (Yoshida and Fujiwara, unpublished result).

Fig. 2

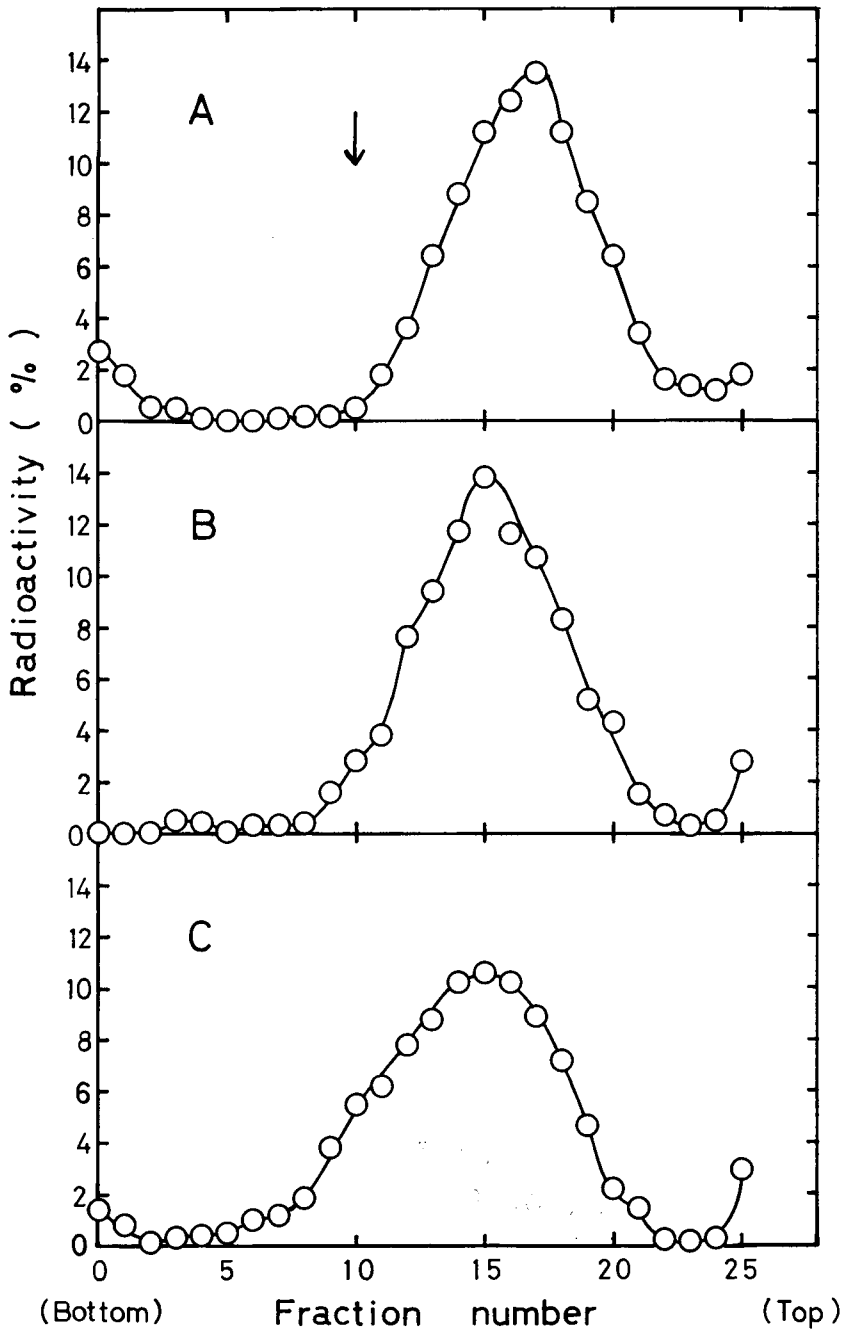
A
B
C
Radioactivity (%)
0
2
4
6
8
10
12
14
0
5
10
15
20
25
(Bottom)
Fraction number
(Top)

Fig. 2 Effect of pre- and post-treatment with araC on SSB rejoining. Prelabelled HeLa cells were exposed to 20 krad. AraC (10^{-5}M) was present for 30 minutes before and for 30 and 60 minutes after irradiation. A, pretreated for 30 minutes, irradiated, lysed immediately; B, pretreated for 30 minutes, irradiated, repair-incubated in araC for 30 minutes before lysis; C, pretreated, irradiated, incubated in araC for 60 minutes before lysis. Arrow indicates the peak position of control DNA.

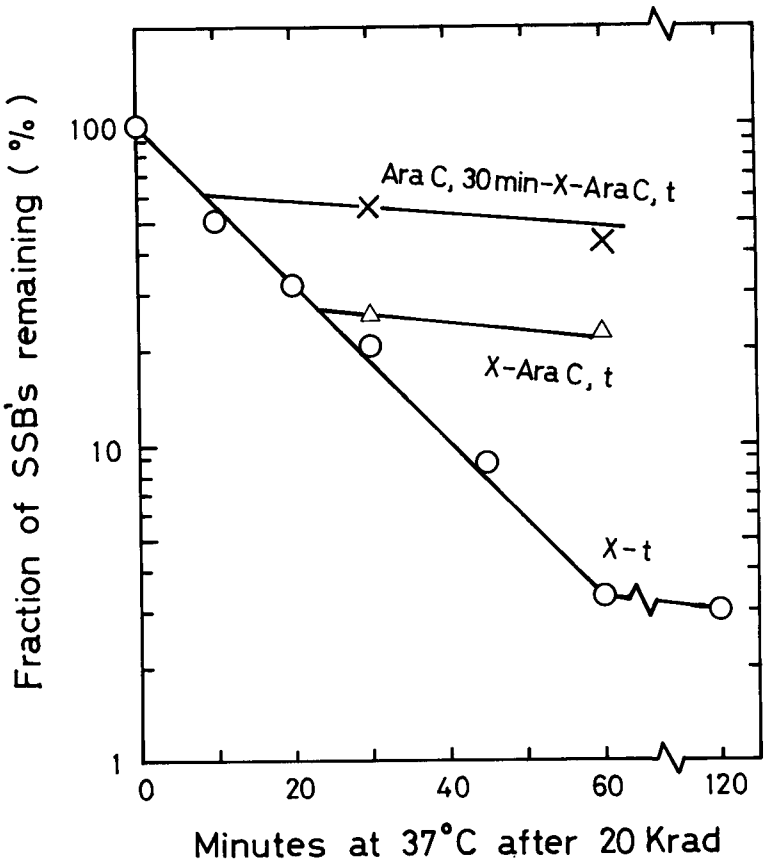

Fig. 3 The kinetics of SSB repair and araC effect. The *Mw* and *Mn* values from sedimentation profiles (Figs. 1, 2 and the data not shown) and the *N* and *f(t)* values were calculated as described in MATERIALS AND METHODS. The ordinate is the plots of *f(t) vs* repair time *(t)* at 37°C. —o—, X-*t*;——Δ—— , X-araC, *t*; ——x——, araC, 30 minutes-X-araC, *t*.

Partial Inhibition of Repair Replication by AraC.

HeLa S3 cells given 0 or 20 krad were immediately incubated in 10^{-5} M [^{3}H]BUdR plus HU for 4 hours (see *labeling protocol (b)*). It is known that HU selectively inhibits semiconservative replication, but does not repair replication.[16] DNA was extracted and 50 μg in a tube was first centrifuged in neutral CsCl. The DNA in the light band at 1.700 g/ml (data not shown) was pooled and dialysed. Then, the same amount of DNA, 33 μg/ml, was rebanded in alkaline CsCl. Fig. 4 shows the alkaline CsCl profiles. X-ray (20 krad) obviously induced repair synthesis, since the majority of radioactivity is associated with light single-stranded DNA at a density of 1.723 g/ml. On the basis of the similar A_{260} profile (Fig. 4, solid line), the presence of araC for 30 minutes before and for 4 hours after 20 krad prevented repair replication partially. Thus, araC inhibition of SSB repair is in part related to its partial inhibition of repair synthesis.

Effects of AraC on Elkind's SLD Recovery.

SLD recovery is characterized by reappearance of shoulder of survival curve during incubations between dose fractions.[3,4,5,6] Further, the maximum initial recovery is attained in 1.5 to 4 hours following the first dose.[3,4,5,6,18,19,20]

Fig. 5 shows SLD recovery of HeLa S3 cells and effects of araC. In *Curve A* (single dose survival), the values of Do (mean lethal dose) and n (extrapolation number) are 180 rad and 1.5, respectively. In other words, the fractionated dose survival curve at 0 hour after 240 rad has no shoulder with n = 1 (*Curve A* in the inserted pannel). *Curve B* indicates the cell fractions surviving serial dose fractions given at the 2 hours' interval following the first 240 rad, where a shoulder reappeared (n = 1.4). (The change in Do was a little from 180 to 230 rad.) Thus, HeLa S3 cells perform SLD recovery. *Curve C* shows the effect of araC present for 30 minutes before and for 2 hours after the first 240 rad. In this instance, the cells were reversed by addition of excess CdR immediately after the second serial doses. Incidentally, it should be noticed that the identical araC treatment and CdR reversal exerted no appreciable toxicity to unirradiated control cells. Characteristically, *Curve C* lost its shoulder (n = 1.0), indicating that araC prevents SLD repair without remarkable effect on the Do value. Fig. 6 displays the cross-

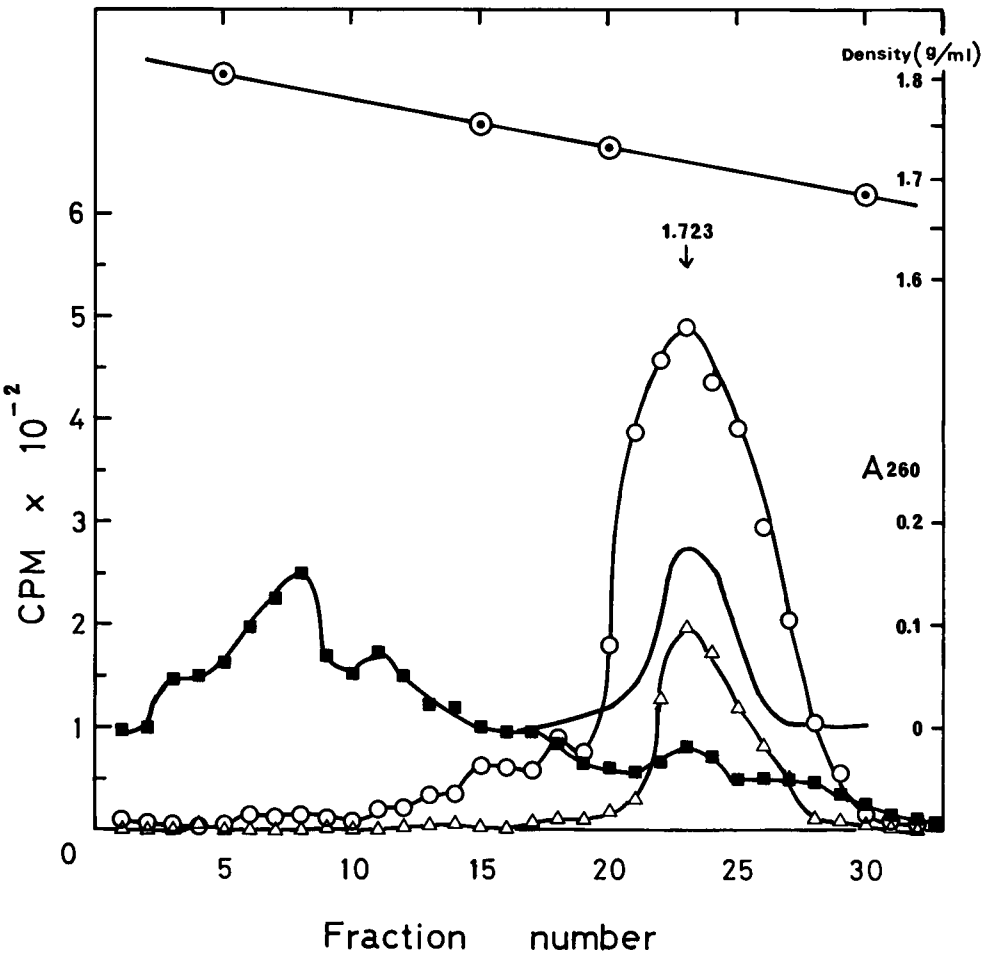

Fig. 4 [^{3}H]BUdR repair incorporation into X-irradiated HeLa S3 cells. The cells pretreated with FUdR plus HU were exposed to 0 or 20 krad at 0°C and repair-incubated in [^{3}H]BUdR plus FUdR and HU for 4 hours. DNA was extracted, and 50 μg of DNA per tube was first banded in neutral CsCl. The DNA band at 1.700 g/ml was pooled and dialysed, followed by rebanding (33 μg/tube) in alkaline CsCl (see the details in MATERIALS AND METHODS). ——■——, control DNA; ——○—— , X-irradiated DNA, ——△—— , DNA from the araC(30 minutes)-X-araC (4 hours) protocol; ——— , A_{260}.

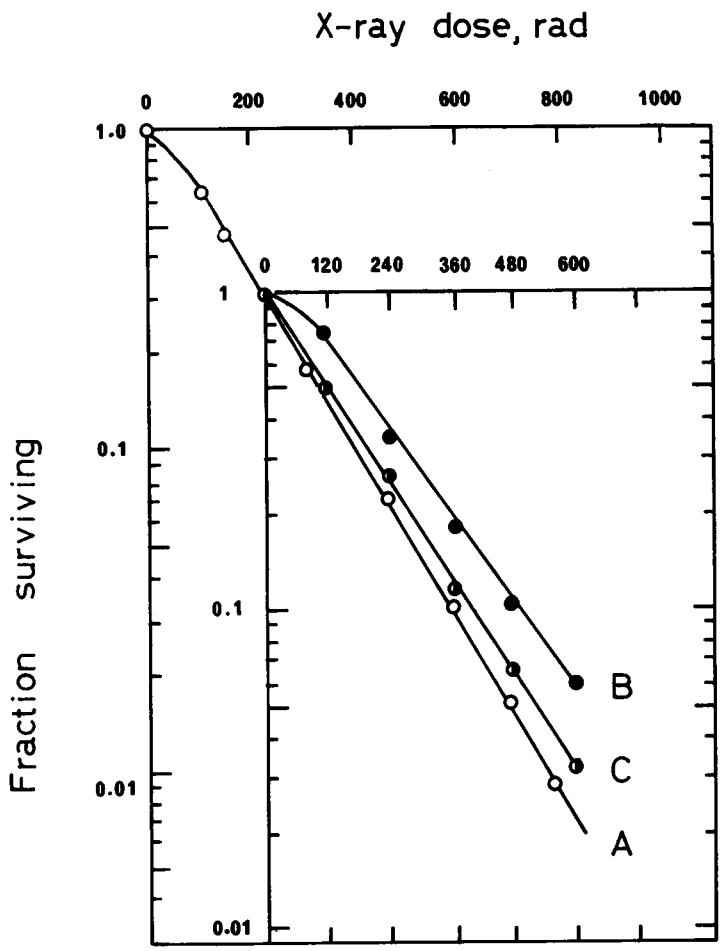

Fig. 5 SLD recovery and its inhibition by araC, as revealed by fractionated dose survival. Log-phase HeLa S3 cells were exposed to the first 240 rad, followed by the second serial doses at a 2 hours' interval. *Curve A* (———○———), single dose survival curve (n = 1.5, Do = 180 rad); *Curve B* (———●———), 240 rad-2 hours-serial doses (n = 1.4, Do = 230 rad); *Curve C* (———◑———), araC, 30 minutes-240 rad-araC, 2 hours-serial doses-CdR (n = 1.0, Do = 210 rad).

section of SLD recovery as a function of time between two 240 rads. Expected full recovery was attained by 1.5 to 2 hours after the first dose (the 240 rad-*t*-240 rad protocol), and then the cells progressed to a sensitive phase, as agreed with the first Elkind's result with V79 Chinese hamster cells.[4] A 30 minutes' araC pretreatment and an immediate CdR addition after the first

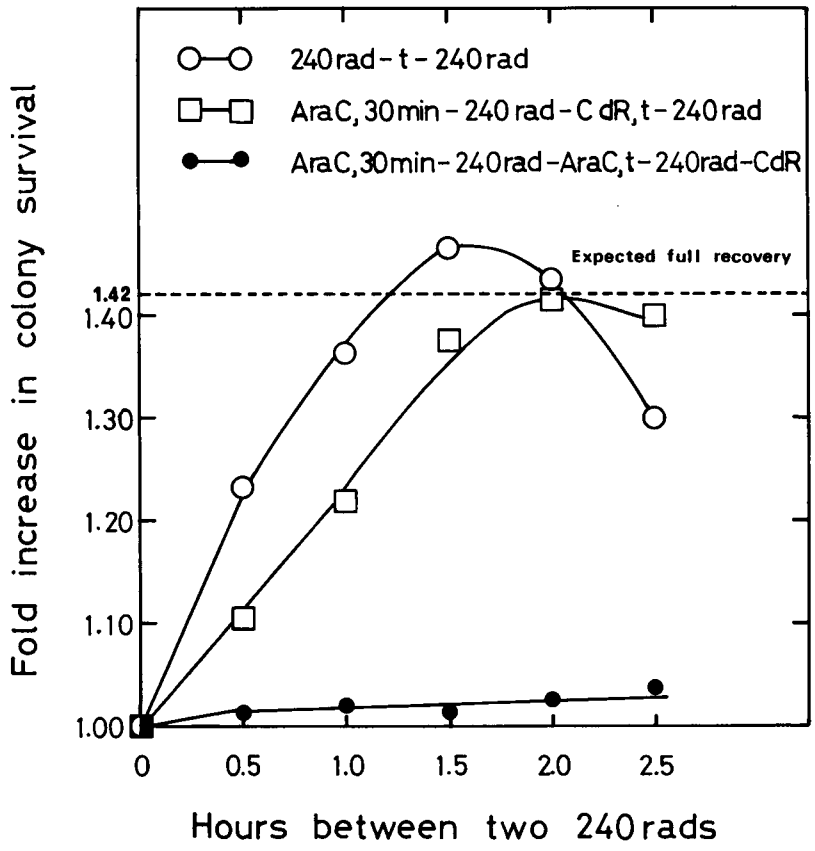

Fig. 6 SLD recovery as a function of time between two 240 rads. Survival after the expected full recovery at 2 hours between two 240 rads and the single 480 rad survival are 0.0993 (0.315 x 0.315) and 0.070, respectively, from Fig. 5 so that the level of expected full recovery is 0.0993/0.070 = 1.42, as shown by the broken line.

240 rad delay simply the initial SLD recovery process (the araC, 30 minutes-240 rad-CdR, *t*-240 rad protocol). The interesting finding is that the araC, 30 minutes-240 rad-araC, *t*-240 rad, CdR protocol alone inhibited SLD recovery successfully and completely. The latter result is consistent with *Curve C* in Fig. 5.

DISCUSSION

The results here have demonstrated that araC suppresses X-ray induced SSB repair possibly requiring repair replication and the initial SLD recovery process. It has been long obscure whether or not SSB repair is responsible for SLD recovery. Low concentrations of actinomycin D (0.005 to 0.05 μg/ml) present between the two dose fractions prevent SLD recovery efficiently[6] and also inhibit repair of a DNA complex,[2] but do not affect SSB repair itself.[2] Further, the time-dose interrelation between SSB and SLD is thought not to be consistent in the aspects of their production and repair. A new finding here is, therefore, a strong suggestion that SSB repair is at least in part involved in Elkind's SLD recovery. Nevertheless, the possibilities follow that more than just a SSB repair, for example, recovery of cell-surface charge lost by radiation[17] and repair of a DNA complex structure dissociated by radiation[2] may also be required to achieve the full recovery of SLD.

Fig. 6 has confirmed the original recovery model put forward by Elkind et al.:[3,4,5] the kinetics of SLD recovery result from a true restoration from SLD combined with cell progression from a response state to another through the cell cycle. AraC has an effectively reducing effect on rise of the initial recovery curve (Fig. 6). Even at the full recovery time of 2 hours after the first dose, araC completely abolishes the reappearance of a shoulder of the fractionated dose survival curve (Fig. 5). Therefore, this araC effect is not simply ascribed to a cell progression to a sensitive phase, but is a true suppression of the initial recovery process, since araC is not a radiation sensitizer due to no appreciable change in the Do value (Fig. 5, *Curve C*).

The metabolic conversion of araC to araCTP inhibits DNA polymerases.[12] As well, its continuous presence before and after X-irradiation prevents in part repair synthesis requiring repair polymerase(s) (Fig. 4). This partial effect is presumed to be due to small patch repair. This incompleteness and a partial presence of ligase-type repair reflect a partial prevention of SSB repair (Fig. 3). This kind of araC effect is unique and different from that of HU which allows normal repair synthesis, but not normal replication.[16] The SLD recovery inhibitors so far known are

only the DNA binding agents (see Introduction). As a new category, the non-DNA binding agent such as araC should be newly added to the SLD recovery inhibitors.

The nature of biochemical and biophysical processes responsible for SLD recovery has yet to be elucidated, but it is apparent that this process may play an important role in determining the response of both normal and tumorous cells to fractionated doses of low LET radiation.[1,21] The present partial characterization of SLD recovery as molecular events by inactivating both repair and recovery by araC must shed further light on the combined chemo-radiotherapy of malignancies.

ACKNOWLEDGEMENTS

I wish to thank Prof. Y. Fujiwara, Department of Radiation Biophysics, for his supervision and advice to achieve the entire thesis work and his critical reading of the manuscript, and Prof. S. Kimura, Department of Radiology, for his advice from the radiation oncology aspect.

REFERENCES

1. Elkind, M.M. Radiology. 1960. 74. 529/541. Cellular aspects of tumor therapy.
2. Elkind, M.M., and Chang-Liu, C.M. Int. J. Radiat. Biol. 1972. 22. 313/324. Actinomycin D inhibition of repair of a DNA complex from Chinese hamster cells.
3. Elkind, M.M., and Sinclair, W.K. Curr. Top. Radiat. Res. 1964. 1. 165/220. Recovery in X-irradiated mammalian cells.
4. Elkind, M.M., and Sutton, H. Radiat. Res. 1960. 13. 556/593. Radiation response of mammalian cells grown in culture. I. Repair of X-ray damage in surviving Chinese hamster cells.
5. Elkind, M.M., Sutton, H., and Moses, W.B. J. Cell Physiol. 1961. 58 (Suppl. 1). 113/134. Postirradiation survival kinetics of mammalian cells grown in culture.
6. Elkind, M.M., Whitmore, G.F., and Alescio, T. Science. 1964. 143. 1454/1457. Actinomycin D: suppression of recovery in X-irradiated mammalian cells.

7. Fujiwara, Y. Cancer Res. 1975. 35. 2780/2789. Postreplication repair of alkylation damage to DNA of mammalian cells in culture.
8. Fujiwara, Y. Biophys. J. 1975. 15. 403/415. Postreplication repair of ultraviolet damage to DNA, DNA-chain elongation and effects of metabolic inhibitors in mouse L-cells.
9. Fujiwara, Y., Higashikawa, T., and Tatsumi, M. J. Cell. Physiol. (in press.) A retarded rate of DNA replication and normal level of DNA repair in Werner's syndrome fibroblasts in culture.
10. Fujiwara, Y., and Kondo, T. Biochem. Pharmacol. 1973. 22. 323/333. Strand-scission of HeLa cell DNA by bleomycin in vitro and in vivo.
11. Fujiwara Y., and Tatsumi, M. Mutat. Res. 1976. 37. 91/110. Replicative bypass repair of ultraviolet damage to DNA of mammalian cells: caffeine sensitive and caffeine resistant mechanisms.
12. Furth, J.J., and Cohen, S.S. Cancer Res. 1968. 28. 2061/2067. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1-β-D-arabinofuranosyl cytosine and the 5'-triphosphate of 9-β-D-arabinofuranosyl adenine.
13. Hahn, G.M., and Little, L.B. Curr. Top. Radiat. Res. 1972. 8. 39/83. Plateau phase cultures of mammalian cells: An in vitro model for human cancer.
14. Ham, R.G. Exp. Cell Res. 1963. 29. 515/526. An improved nutrient solution for diploid Chinese hamster and human cell lines.
15. Painter, R.B. Radiation research-biomedical, chemical and physical perspectives, ed. by Nygaad, D.F., Adler, H.I., and Sinclair, W.K. Academic Press, N.Y. 1975. 735/739. Chemical changes induced in DNA by ionizing radiation and the relationship of their repair to survival of mammalian cells.
16. Painter, R.B., and Cleaver, J.E. Radiat. Res. 1969. 37. 451/466. Repair replication, unscheduled DNA synthesis and the repair of mammalian DNA.
17. Sato, C., Kojima, K., Matsuzawa, T., Sairenji, T., and Hinuma, Y. J. Radiat. Res. 1974. 15. 25/31. Lack of recovery from radiation injury on colony forming ability and on membrane charge in a Burkitt lymphoma cell line.

18. Sinclair, W.K., and Morton, A.R. Nature. 1964. 203. 247/250. Recovery following X-irradiation of synchronized Chinese hamster cells.
19. Terasima, T. Recovery from radiation damage, ed. by Egami, N. Asakura Shoten, Tokyo. 1970. 84/102. Radiation damage and recovery in higher animal cells (in Japanese).
20. Terasima, T. and Yasukawa, M. Comparative cellular and species specificity, ed. by Sugahara, T., and Bond, V.P. Igaku Shoin Publ. Co., Tokyo. 1969. 79/86. Effects of some metabolic inhibitors on the recovery in X-irradiated mouse L cells.
21. Withers, H.R. Adv. Radiat. Biol. 1976. 5. 241/271. The four R's of radiotherapy.