# 1-β-D-Arabinofuranosylcytosine Is Cytotoxic in Quiescent Normal Lymphocytes Undergoing DNA Excision Repair

Takahiro Yamauchi, Yasukazu Kawai and Takanori Ueda

*First Department of Internal Medicine, Fukui Medical University, 23 Shimoaizuki, Matsuoka, Fukui 910-1193*

We have sought to clarify the potential activity of the S-phase-specific antileukemic agent 1-β-D-arabinofuranosylcytosine (ara-C), an inhibitor of DNA synthesis, in quiescent cells that are substantially non-sensitive to nucleoside analogues. It was hypothesized that the combination of ara-C with DNA damaging agents that initiate DNA repair will expand ara-C cytotoxicity to non-cycling cells. The repair kinetics, which included incision of damaged DNA, gap-filling by DNA synthesis and rejoining by ligation, were evaluated using the single cell gel electrophoresis (Comet) assay and the thymidine incorporation assay. When normal lymphocytes were treated with ultraviolet C or with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), the processes of DNA excision repair were promptly initiated and rapidly completed. When the cells were incubated with ara-C prior to irradiation or BCNU treatment, the steps of DNA synthesis and rejoining in the repair processes were both inhibited. The ara-C-mediated inhibition of the repair processes was concentration-dependent, with the effect peaking at 10 $\mu M$. The combination of ara-C with these DNA repair initiators exerted subsequent cytotoxicity, which was proportional to the extent of the repair inhibition in the presence of ara-C. In conclusion, ara-C was cytotoxic in quiescent cells undergoing DNA repair. This might be attributed to unrepaired DNA damage that remained in the cells, thereby inducing lethal cytotoxicity. Alternatively, ara-C might exert its own cytotoxicity by inhibiting DNA synthesis in the repair processes. Such a strategy may be effective against a dormant subpopulation in acute leukemia that survives chemotherapy.

Key words:    ara-C — DNA repair — UV — BCNU — Comet assay

1-β-D-Arabinofuranosylcytosine (ara-C) is one of the most effective anticancer agents for the treatment of leukemia and lymphoma.[1, 2] It is phosphorylated in the cell to its active metabolite, 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP), which inhibits DNA polymerases in competition with deoxycytidine 5′-triphosphate.[3–5] Intracellular ara-CTP is in part incorporated into DNA, thereby terminating DNA elongation.[6] Thus, the essential cytotoxicity of ara-C is attributed to inhibiting DNA synthesis, and it is, therefore, specific to the S-phase in growing cells.[3–6]

The cytotoxic effects of ara-C are substantially limited because only a small population of tumor cells is in the active cell cycle.[7] The drug would be non-toxic to slow-growing tumors such as chronic lymphocytic leukemia lymphocytes and to non-cycling cells, including a dormant subpopulation in acute myeloid leukemia cells. These malignant cells would survive the chemotherapy, leading to treatment failure.

To extend the activity of ara-C to non-cycling cells, DNA repair may be manipulated because several studies have demonstrated that ara-C inhibits the repair process.[8–11] If ara-C is combined with DNA-damaging agents that ini-

tiate DNA repair, ara-C would inhibit the repair process, and the unrepaired DNA damage would induce lethal toxicity in the cells. Alternatively, as the repair process includes unscheduled DNA synthesis for gap-filling after removal of the damaged nucleotides,[12–14] ara-C would be cytotoxic by inhibiting the DNA synthesis. Thus, it is hypothesized that ara-C can be cytotoxic to non-cycling cells in the context of DNA repair.

The previous studies have demonstrated the precise kinetics of nucleotide excision repair in quiescent normal lymphocytes irradiated with ultraviolet C (UV) using the single cell gel electrophoresis (Comet) assay.[15, 16] Non-cycling lymphocytes could respond to UV-induced DNA damage by initiating nucleotide excision repair. Pre-treatment with fludarabine was inhibitory to the repair process. The present investigation was prompted by these findings.

In the present study, we investigated the activity of ara-C on non-cycling normal lymphocytes undergoing DNA repair. UV and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a bifunctional alkylating agent, were used to initiate DNA excision repairs. Alkylating agents would be more clinically relevant than UV with regard to treatment of hematological malignancies. While UV induces nucleotide excision repair exclusively, BCNU induces several excision repairs such as base excision repair, nucleotide

E-mail: tyamauch@fmsrsa.fukui-med.ac.jp

excision repair, and cross-link repair.[12–14, 17, 18] The processes of such excision repairs include the incision and excision of the damaged nucleotides, DNA resynthesis for gap-filling, and rejoining by ligation. Therefore, the Comet assay and the thymidine incorporation assay were employed to quantitate the kinetics of the repairs, as previously described.[16] The subsequent effect on cell viability was determined as apoptotic cell death by nuclear morphology using Hoechst staining or by externalization of phosphatidylserine using FITC-conjugated Annexin V.[19, 20]

## MATERIALS AND METHODS

**Chemicals and reagents**　ara-C and BCNU were purchased from Sigma (St. Louis, MO). BCNU was dissolved in 100% ethanol immediately before use. [Methyl,1′,2′-$^3$H]thymidine (123 Ci/mmol) was purchased from Amersham International (Buckinghamshire, UK).

**Lymphocyte preparation**　Normal lymphocytes from 5 healthy donors were used. Whole blood was drawn into heparinized tubes, layered over Ficoll-Paque solution, and centrifuged at 1500 rpm for 30 min.[21] The lymphocytes were harvested from the interphase, washed twice with phosphate-buffered saline (PBS), and resuspended at $1 \times 10^6$ cells/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Life Technologies, Inc., Grand Island, NY) and 2 m$M$ L-glutamine. The cells were incubated in a 5% $CO_2$-humidified atmosphere at 37°C overnight.

**UV exposure and drug treatments**　Lymphocytes ($1 \times 10^6$ cells/ml in 2 ml) were pre-incubated for 2 h with ara-C at various concentrations or not, followed by a 5 J/m$^2$ UV exposure or by incubation with BCNU at various concentrations for the indicated periods. The cells were immediately followed by washing in fresh media and subsequent incubation for the indicated periods.

**Evaluation of DNA repair kinetics**　To evaluate the kinetics of DNA excision repair, the amount of DNA single-strand breaks was quantitated, because they were generated by the incision and were diminished by the rejoining in the repair process. The Comet assay was used for this purpose according to the method previously described, with a slight modification.[16] Briefly, lymphocytes after treatments were mixed with agarose and placed onto a microscope slide. After solidification of the agarose, the slide was left in a lysis solution (2.5 $M$ NaCl, 10 m$M$ Tris, 100 m$M$ ethylenediaminetetraacetic acid, 10% dimethylsulfoxide, and 1% Triton X-100, pH 10) for 1 h at 4°C. The slide was then soaked in an alkaline buffer (1 m$M$ ethylenediaminetetraacetic acid, and 300 m$M$ NaOH, pH 13) for 30 min, and subjected to electrophoresis (35 V, 300 mA) for the next 30 min at 4°C. After the electrophoresis, the slide was neutralized and stained with ethidium bromide. Cells, 100 per treatment condition, were

analyzed using a computer-based image analysis system (Kinetic Imaging Komet system, Version 4.0, Liverpool, UK). Single-strand breaks were quantitated and expressed as the "tail moment," which combines a measurement of the distance of the DNA migration and the relative DNA content therein.[22]

**Evaluation of unscheduled DNA synthesis**　To determine DNA resynthesis for gap-filling during the repair (unscheduled DNA synthesis), the incorporation of tritiated thymidine into DNA was measured as previously described.[15, 16] In brief, normal lymphocytes ($2 \times 10^6$ cells) having previously been treated and washed in fresh media were incubated with tritiated thymidine (3 $\mu$Ci/ml) for 0, 1, 2, 4, and 6 h. The lymphocytes were then collected, centrifuged, and resuspended in 500 $\mu$l of 0.4 $N$ perchloric acid. The sample was mixed, centrifuged, and resuspended again in the acid. After further mixing and centrifugation, the pellet was resuspended in 1 ml of 0.5 $N$ KOH and incubated at 45°C overnight to dissolve the pellet. Radioactivity was counted on the following day.

**Quantitation of apoptotic cell death**　To evaluate the subsequent cytotoxicity following the repair inhibition, apoptotic cell death was determined in terms of nuclear morphology or phosphatidylserine externalization at 24 h after the treatments.[19, 20] Normal lymphocytes having been treated were incubated with 2 $\mu$g/ml Hoechst No. 33342 for 30 min at 37°C. Nuclei, 200 per treatment condition, were counted under UV illumination. Apoptotic cell death was determined by nuclear morphology with nuclear condensation and fragmentation.

Alternatively, normal lymphocytes after the treatments were collected by centrifugation (1500 rpm, 5 min) and resuspended in 200 $\mu$l of binding buffer (10 m$M$ Hepes/NaOH, pH 7.4, 140 m$M$ NaCl, and 2.5 m$M$ CaCl$_2$) and 10 $\mu$l of FITC-conjugated Annexin V. The sample was incubated for 15 min at room temperature and centrifuged again. The pellet was resuspended with 500 $\mu$l of the same buffer and with 10 $\mu$l of 50 $\mu$g/ml propidium iodide. The samples were analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Data acquisition and analysis were performed by the CellQuest program (Becton Dickinson). Cells positive for Annexin V were considered apoptotic.

## RESULTS

**ara-C inhibited nucleotide excision repair**　First, to determine the effect of ara-C on nucleotide excision repair, the Comet assay was conducted to measure the amounts of DNA single-strand breaks generated through the repair process (Fig. 1). When normal lymphocytes were irradiated, the tail moment was greatest at 1 h after the exposure and decreased promptly thereafter. This suggests that the DNA strand breaks resulting from the incision step peaked

at 1 h and were rapidly rejoined in the repair process. The tail-moment value came to the control level at 4 h, indicating the completion of the repair process.

When the lymphocytes were pre-incubated with 10 $\mu M$ ara-C followed by UV irradiation, the tail-moment values at 4 h after the exposure were greater than the value generated by UV alone (Fig. 1). This high tail moment was maintained and did not return to the control value, suggesting the inhibition of the rejoining step in the process of nucleotide excision repair. The tail-moment value at 1 h

was unchanged by the pre-incubation with ara-C, suggesting that ara-C had no effect on the incision step. These results are broadly compatible with those demonstrated by the combination treatment of UV with fludarabine in the previous study.[16]

**Concentration-dependent inhibition of repair** To further evaluate ara-C-mediated inhibition of repair, lymphocytes were pre-incubated with various concentrations of ara-C or not, followed by 5 J/m$^2$ UV irradiation and by washing in fresh media. The tail-moment values at 4 h after the exposure became greater log-linearly as the concentration of ara-C increased (Fig. 2A), but appeared to reach a plateau at 10 $\mu M$ (Fig. 2B). The uptake of thymidine into DNA, which represents unscheduled DNA synthesis, was also inhibited by ara-C with the effect peaking at 10 $\mu M$ (Fig. 2C). These results suggest that ara-C inhibited the steps of both DNA resynthesis and rejoining in a concentration-dependent manner, and that 10 $\mu M$ would be a target concentration to obtain the maximal inhibition of repair.

**ara-C was cytotoxic to normal lymphocytes undergoing nucleotide excision repair** To see if ara-C was cytotoxic to non-cycling lymphocytes exposed to UV, apoptotic cell death was determined in terms of nuclear morphology (Fig. 3) at 24 h after lymphocytes had been treated with ara-C, or with UV, or with both in combination. Although 20 $\mu M$ ara-C was non-toxic to lymphocytes, the combination of ara-C with a minimally toxic dose (5 J/m$^2$) of UV produced more-than-additive cytotoxicity compared with the sum of each treatment alone (Fig. 4A). This suggests that ara-C can be active in quiescent cells undergoing nucleotide excision repair. The cytotoxicity was enhanced with increasing concentration of ara-C (Fig. 4B), and the increase in the cytotoxicity was proportional to the tail-



Fig. 1. ara-C inhibited nucleotide excision repair. Normal lymphocytes from 5 healthy donors were pre-incubated with 10 $\mu M$ ara-C or not, followed by 5 J/m$^2$ UV exposure. The samples were then washed and resuspended in fresh media. Tail moments were determined by the Comet assay at 0, 0.5, 1, 2, 4, 6, and 8 h after resuspension. ○, UV exposure alone (mean±SD); ●, pre-incubation with 10 $\mu M$ ara-C, followed by UV exposure (mean±SD). The tail-moment value of untreated cells was set as a control (hair line).
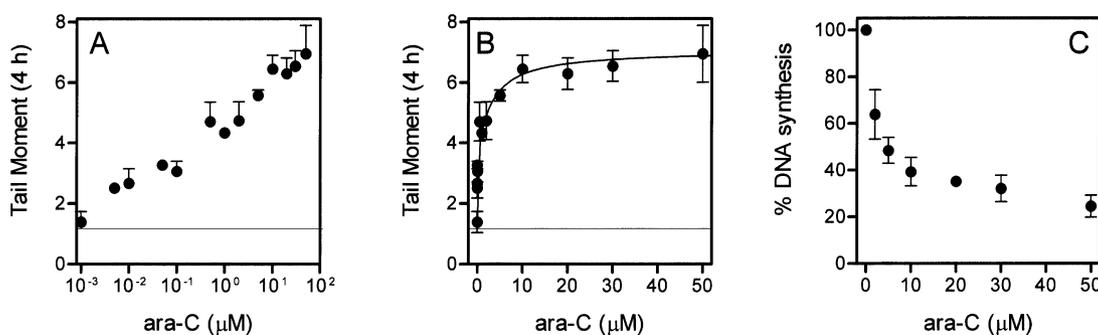


Fig. 2. Concentration-dependent inhibition of the repair. Normal lymphocytes from 5 healthy donors were pre-incubated for 2 h with various concentrations (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 30, and 50 $\mu M$) of ara-C or not, followed by 5 J/m$^2$ UV exposure and by washing the cells in fresh media. Tail moments were determined at 4 h after the washing (mean±SD) (A, semi-log; B, linear), whereas the radioactivities of the cellular DNA were measured at 6 h after incubation with tritiated thymidine following the washing (mean±SD) (C). Percent DNA synthesis was defined as the relative value (%) of the radioactivity for the combination treatment to that for UV alone.

moment value at 4 h generated in the presence of ara-C at the respective concentration (Fig. 4C). These results suggest a close association between the inhibition of repair and the subsequent cytotoxicity.

**Incision response to BCNU** Next, BCNU was used as a DNA repair initiator. BCNU induces not only nucleotide excision repair, but also base excision repair and cross-link repair. To confirm that DNA single-strand breaks were generated through the cellular response to BCNU, normal lymphocytes were incubated with various concentrations of BCNU for 30 min or with 60 $\mu M$ BCNU for various time periods. The tail-moment values were increased both concentration- and time-dependently (Fig. 5), suggesting an enhanced incision reaction corresponding to the increased BCNU-induced DNA damage. The curve was most linear between 40 and 100 $\mu M$ BCNU (Fig. 5A) or

between 15 and 45 min of incubation (Fig. 5B). Therefore, the subsequent experiments were performed using a 30-min incubation period with 60 $\mu M$ BCNU.

**ara-C inhibited BCNU-induced excision repair** To determine the kinetics of excision repair initiated by BCNU, normal lymphocytes having been treated with BCNU were subjected to Comet assay at the indicated time points. The tail moment was greatest at the end of the incubation period (tail moment at 0 h), suggesting that the incision reaction was maximal at this point (Fig. 6A). Thereafter, the tail moment rapidly decreased and returned to the control level at 4 h, suggesting that the incised DNA was rejoined by ligation, and the repair process was com-
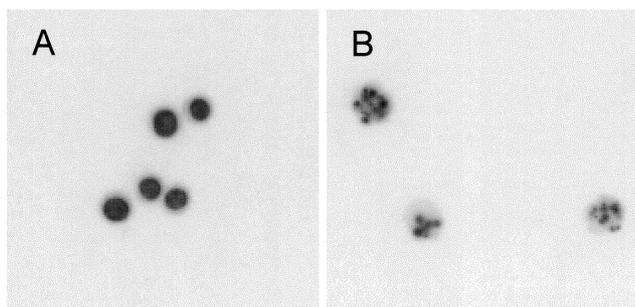


Fig. 3. Typical morphologic features of apoptotic nuclei stained using Hoechst No. 33342. (A) Untreated lymphocytes. (B) Lymphocytes treated with the combination of ara-C and UV showing nuclear fragmentation.
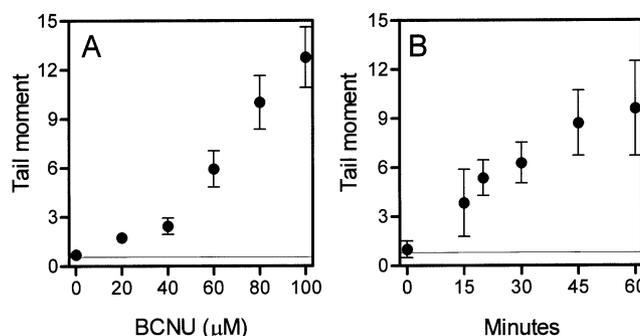


Fig. 5. Incision response to BCNU. Normal lymphocytes from 3 healthy donors were incubated for 30 min with various concentrations (0, 20, 40, 60, 80, or 100 $\mu M$) of BCNU (A), or with 60 $\mu M$ BCNU for various time periods (15, 20, 30, 45, and 60 min) (B) (mean±SD). The tail moments were measured at the end of the incubation period. The tail-moment value of the untreated cells was set as a control (hair line).
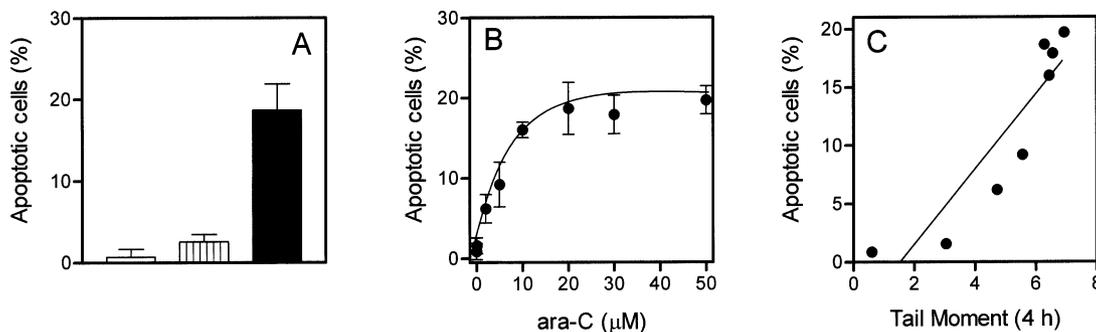


Fig. 4. ara-C was cytotoxic to normal lymphocytes undergoing nucleotide excision repair. Normal lymphocytes from 5 healthy donors were treated with a 2-h incubation with various concentrations (0.1, 2, 5, 10, 20, 30, and 50 $\mu M$) of ara-C, or with 5 J/m² UV exposure, or with both in combination. Apoptotic cell death was determined in terms of nuclear morphology using Hoechst No. 33342 staining at 24 h after the treatments. (A) ara-C (20 $\mu M$) combined with UV increased the cytotoxicity (mean±SD). □, UV 5 J/m²; ▥, ara-C 20 $\mu M$; ■, combination. (B) Apoptotic cell death increased as the concentration of ara-C was increased (mean±SD). (C) The correlation between the tail-moment value and the subsequent apoptotic cell death. The tail-moment values at 4 h for various concentrations of ara-C (the mean values from Fig. 2A) were proportional to the subsequent increases in apoptotic cell death (the mean values of Fig. 4B). $R=0.8$, $P=0.02$.

pleted. The concentration (60 $\mu M$) of BCNU used here was higher than the plasma concentration (5–10 $\mu M$) usually achieved in the clinic.[23] However, it was considered to be appropriate for the present experimental setting using normal lymphocytes, as the cells were capable of repairing the damage.
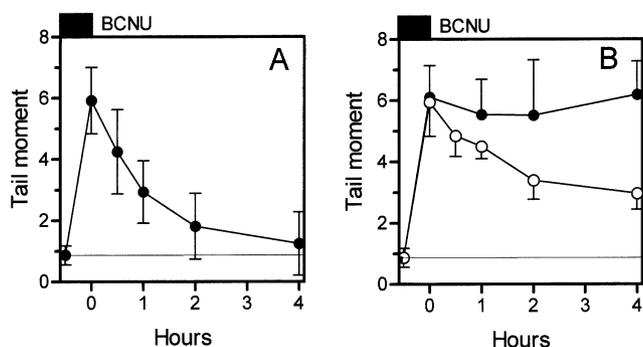


Fig. 6.   ara-C inhibited BCNU-induced excision repair. Normal lymphocytes from 5 healthy donors were pre-incubated for 2 h with ara-C (B) or not (A), followed by a 30-min incubation with 60 $\mu M$ BCNU. Tail moments were determined by the Comet assay at 0, 0.5, 1, 2, and 4 h after the samples had been washed and resuspended in fresh media. (A) Kinetics of BCNU-induced excision repair (mean±SD). (B) The inhibition of BCNU-induced excision repair by ara-C (mean±SD). ○, pre-incubation with 5 $\mu M$ ara-C, followed by BCNU (mean±SD); ●, pre-incubation with 10 $\mu M$ ara-C, followed by BCNU (mean±SD). The tail-moment value of untreated cells was set as a control (hair line).

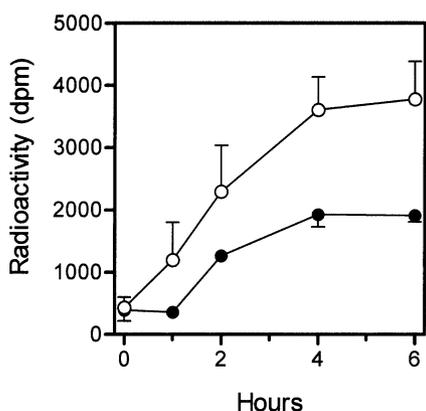When the lymphocytes were pre-incubated with ara-C prior to BCNU treatment, the tail-moment value at 4 h after the end of the incubation period was greater than the value generated by BCNU alone (Fig. 6B). This suggests the inhibition of the rejoining step in the excision repair process. The tail-moment value at 4 h generated by pre-incubation with 10 $\mu M$ ara-C was greater than that with 5 $\mu M$ ara-C, suggesting greater inhibition of the rejoining by the higher concentration. The tail-moment value at 0 h was unchanged by ara-C-pre-treatment, suggesting that ara-C had no effect on the incision reaction. Thus, ara-C inhibited BCNU-induced excision repair in a similar way to that found in the context of UV.

**Unscheduled DNA synthesis**   To evaluate the DNA resynthesis step, the thymidine incorporation assay was performed. After the lymphocytes had been treated with BCNU, the uptake of thymidine into DNA was increased and appeared to reach a plateau at 4 h (Fig. 7). This suggests that unscheduled DNA synthesis was initiated by BCNU, and the process was completed within 4 h. Pre-treatment with ara-C significantly reduced the uptake ($P=0.03$, for the 6-h values between ara-C+BCNU and BCNU alone, evaluated by a paired $t$ test), suggesting the inhibition of the DNA resynthesis step.

**ara-C was cytotoxic to normal lymphocytes undergoing BCNU-induced excision repair**   To clarify the cytotoxicity of ara-C on quiescent lymphocytes treated with BCNU, apoptotic cell death was determined at 24 h after lymphocytes had been treated with ara-C, or BCNU, or both in combination. The combination provided greater-than-additive cytotoxicity compared with the sum of each drug alone (Fig. 8), suggesting that ara-C could be active in



Fig. 7.   Unscheduled DNA synthesis. Normal lymphocytes from 3 healthy donors were pre-incubated for 2 h with 10 $\mu M$ ara-C (●) or not (○), followed by a 30-min incubation with 60 $\mu M$ BCNU. The samples were then incubated with tritiated thymidine for 0, 1, 2, 4, and 6 h, and the radioactive counts of the cellular DNA were determined (mean±SD).
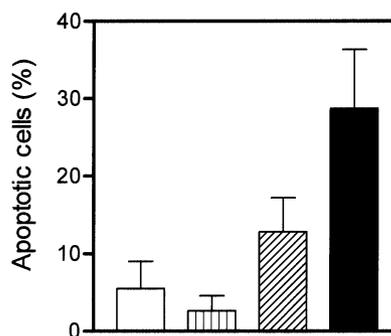


Fig. 8.   ara-C was cytotoxic to normal lymphocytes undergoing BCNU-induced repair. Normal lymphocytes from 5 healthy donors were pre-incubated for 2 h with 5 $\mu M$ ara-C or 10 $\mu M$ ara-C or not, followed by a 30-min incubation with 60 $\mu M$ BCNU. Apoptotic cell death was determined by externalization of phosphatidylserine using Annexin V at 24 h after the cells had been washed and resuspended in fresh media. ☐, BCNU 60 $\mu M$; ▥, ara-C 10 $\mu M$; ▨, ara-C 5 $\mu M$+BCNU 60 $\mu M$; ■, ara-C 10 $\mu M$+BCNU 60 $\mu M$.

quiescent lymphocytes undergoing BCNU-induced excision repair. In addition, the combination with 10 $\mu M$ ara-C was more cytotoxic than that with 5 $\mu M$ ara-C. These results suggest that the higher concentration of ara-C exerted greater inhibition of the repair, thereby inducing more potent cytotoxicity in quiescent lymphocytes.

## DISCUSSION

The present study was conducted to clarify the cytotoxicity of ara-C in quiescent normal lymphocytes. ara-C inhibited the process of DNA excision repair initiated by both UV and BCNU. Subsequently, ara-C combined with these modalities exerted cytotoxicity in the quiescent lymphocytes. The increase in the cytotoxicity appeared correlated to the extent of inhibition of the repair process. Thus, ara-C could be active in non-cycling cells in the context of DNA repair, although ara-C alone was non-toxic.

Pre-treatment with ara-C inhibited UV-induced repair in a concentration-dependent manner (Figs. 1 and 2). This effect was demonstrated by the inhibition of thymidine incorporation and by the retention of the high tail-moment value at 4 h, suggesting that ara-C inhibited mainly the DNA synthesis step and the rejoining step of the repair process (Fig. 2). This would be compatible with the essential activity of ara-C as an inhibitor of DNA synthesis and ligation.[3–6, 8–11]

The increase in the subsequent cytotoxicity by the combination of ara-C with UV was proportional to the tail-moment value at 4 h in the presence of ara-C (Fig. 4C). As ara-C was non-toxic to lymphocytes even at 20 $\mu M$, it is suggested that the cytotoxicity was generated specifically through the inhibition of repair, i.e., it might be attributed to unrepaired DNA damage that remained in the cells, thereby inducing lethal cytotoxicity. Alternatively, ara-C might exert its own cytotoxicity by inhibiting DNA synthesis in the repair process.

Surprisingly, ara-C had some inhibitory effect at the very low concentration of 10 n$M$ (Fig. 2A), suggesting that even low-dose ara-C therapy may inhibit DNA repair in dormant leukemic cells in the clinical setting. On the other hand, 10 $\mu M$ ara-C maximized the inhibitory effect of repair and the subsequent cytotoxicity in lymphocytes exposed to UV (Fig. 4). This concentration is achievable in the plasma by intermediate- to high-dose ara-C administration.[24] Thus, the mechanistic interaction of ara-C with DNA repair described here may be applicable in the clinic.

BCNU was also used as DNA repair initiator. Alkylating agents may be clinically relevant because they are employed in the treatment of hematological malignancies, in contrast to UV. Although BCNU is not used for the therapy of leukemia, it is a bi-functional alkylator, inducing 90% of mono-adducts and 3–5% of interstrand cross-links, which are similar values to those induced by other alkylators.[18, 23] These adducts are subject to four general categories of repair, i.e. direct repair, base excision repair, nucleotide excision repair, and cross-link repair.[25] Although $O^6$-alkylguanine is directly repaired by $O^6$-alkylguanine DNA alkyltransferase,[26] several mono- and di-adducts, including crucial interstrand cross-links, are removed by excision repairs. Therefore, DNA excision repair initiated by BCNU can be a model for evaluating combination strategies with ara-C against leukemia.

When normal lymphocytes were treated with BCNU, DNA single-strand breaks were generated concentration- and time-dependently, suggesting that the incision was generated in response to BCNU (Fig. 5). When the cells were pulsed with BCNU, the tail-moment value was greatest at the end of the incubation period, suggesting that the incision reaction peaked rapidly (Fig. 6A). The uptake of thymidine, representing unscheduled DNA synthesis, reached a plateau at 4 h, suggesting the completion of the gap-filling that followed the incision step (Fig. 7). The tail moment subsequently decreased, suggesting rapid rejoining of incised DNA (Fig. 6A). The reversion of the tail moment to the control level at 4 h represents the completion of the repair process (Fig. 6A). Thus, the whole repair process of DNA excision repair initiated by BCNU was precisely quantitated, and its kinetics was very similar to that of UV-induced nucleotide excision repair.

Pre-treatment of ara-C also inhibited excision repair initiated by BCNU. The effect of ara-C was observed as a reduction of thymidine incorporation into DNA (Fig. 7) and retention of a high tail-moment value at 4 h (Fig. 6B). Moreover, the inhibitory effect was greater at a higher concentration of ara-C (Fig. 6B). These results suggest that ara-C inhibited BCNU-induced repair in a very similar way to that found in the context of UV (Fig. 2).

ara-C combined with BCNU exerted cytotoxicity in normal lymphocytes. A higher concentration of ara-C provided a greater cell killing effect. As ara-C produced greater inhibition of repair at higher concentration, the results suggest a close association between the inhibition of the repair and the subsequent cytotoxicity (Figs. 6B and 8). Also, these results were very similar to those seen in the case of combination treatment of ara-C with UV. Thus, it is suggested that ara-C could be cytotoxic to quiescent lymphocytes in the context of DNA excision repair.

We have manipulated DNA repair to enhance the cytotoxicity of ara-C to non-cycling cells. Previous studies demonstrated that a similar nucleoside analogue, fludarabine, inhibited the process of UV-induced nucleotide excision repair, thereby generating cytotoxicity to normal lymphocytes.[15, 16] However, these reports did not reveal the exact relationship between the inhibition of repair and the subsequent cytotoxicity. The present study has clearly demonstrated that ara-C was active in quiescent lymphocytes undergoing DNA repair, and that the cytotoxic activ-

ity of ara-C was correlated with its inhibitory effect on the repair. Normal lymphocytes could be biological models for investigating the paradigm of quiescence. We speculate that ara-C may be cytotoxic to a dormant subpopulation in acute leukemia under suitable conditions. The use of ara-C in combination with a DNA-damaging agent at an appropriate dose that induces the damage repair response in leukemic cells, but is not cytotoxic to normal lymphocytes, could provide a therapeutic strategy to overcome resistance and improve clinical efficacy.[27]

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