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ENHANCEMENT OF ULTRAVIOLET-INDUCED UNSCHEDULED DNA SYNTHESIS IN HELA CELL LYSATE BY APHIDICOLIN IN VIVO

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SUMMARY: The effect of in vivo treatment of DNA synthesis inhibitors on ultraviolet-induced unscheduled DNA synthesis in HeLa cell lysate was examined. Aphidicolin with or without hydroxyurea enhanced the in vitro unscheduled DNA synthesis significantly, while 2', 3'-dideoxythymidine did not. Accumulation of single strand breaks on DNA in ultraviolet-irradiated cells was observed in the presence of aphidicolin and hydroxyurea.

INTRODUCTION: Among three known DNA polymerases in eukaryotes, DNA polymerase β is believed to be implicated in DNA repair synthesis (1). However, recent studies utilizing selective inhibitors for DNA polymerases have led to conflicting results: several reports described the experimental data which indicate that polymerase β has major role in DNA repair (2-6). On the other hand, a bulk of studies claimed that DNA polymerase α is necessary in some types of DNA repair synthesis (7-11). Since all of the latter group of studies examined the effect of selective inhibitors in in vitro system, it is doubted whether the same phenomenon occurs in vivo or not (12).

In this report, we studied the effect of in vivo treatment of DNA synthesis inhibitors on ultraviolet (UV)-induced unscheduled DNA synthesis in vitro. Our results indicate that aphidicolin inhibits UV-induced unscheduled DNA synthesis not only in vitro but also in vivo.

MATERIALS AND METHODS:
Reagents: Thymidine[6-^{3}H] (5 Ci/mmol) and dTTP[methyl-^{3}H] (50 Ci/mmol) were obtained from Amersham. 2', 3'-Dideoxythymidine (ddThy) was purchased from P-L Biochemicals. Hydroxyurea, arabinofuranosyl cytosine (araCyt) and

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deoxyribonucleoside triphosphates were purchased from Sigma. Aphidicolin was kindly provided by Dr. M. Ohashi, Tokyo Metropolitan Institute of Gerontology.

Cell culture, synchronization and UV irradiation: HeLa S3 cells were maintained, synchronized at G_2-G_1 phase and irradiated with UV light (254 nm emission peak) as described previously (13).

Assay of dTTP[methyl-^{3}H] incorporation in cell lysate: Cell lysate was prepared and incubated with dTTP[methyl-^{3}H] as described previously.

Alkaline sucrose density gradient analysis: DNA strand breaks were assayed as described previously (11).

RESULTS:

In our previous reports, we found that preincubation of the cells with hydroxyurea and araCyt enhanced the incorporation of dTTP[methyl-^{3}H] in response to exposure to UV and MNNG (11, 13). Alkaline sucrose gradient analysis of DNA revealed that combined treatment of hydroxyurea and araCyt inhibits repair synthesis, while the incision of damaged DNA proceeds. It is interesting to know whether we can use any selective inhibitor of DNA polymerase in place of hydroxyurea and araCyt.

Table 1 shows the effect of *in vivo* treatment of DNA synthesis inhibitors on *in vitro* unscheduled DNA synthesis. Exposure of G_2-G_1 phase HeLa cells to 50 J/m^2 UV light induced only small amount of dTTP[methyl-^{3}H] incorporation by itself. When the cells were treated with one of three inhibitors, hydroxyurea, araCyt and aphidicolin, there were some increases of the incorporation, but the amount was still small. ddThy had no enhancing effect. Combination of araCyt or aphidicolin with hydroxyurea induced UV-dependent unscheduled DNA synthesis more than 20 times higher than no drug treatment. On the other hand, ddThy showed no increase of dTTP incorporation even in the case of combined treatment with hydroxyurea.

Figure 1 shows the dose effect of aphidicolin treated *in vivo* on the incorporation of dTTP[methyl-^{3}H] in lysate prepared from UV-irradiated cells. Aphidicolin enhanced *in vitro* unscheduled DNA synthesis in a dose-dependent manner. Then we examined whether UV-irradiated cells accumulated single strand breaks on DNA in the presence of hydroxyurea and aphidicolin. As shown in Fig. 2, a combined treatment of hydroxyurea and aphidicolin shifted UV-irradiated DNA to a lower molecular weight region as a combination of hydroxyurea and

Table 1. Effect of in vivo treatment of DNA synthesis inhibitors on in vitro unscheduled DNA synthesis

Conditions					[^{3}H]dTMP-incorporated (pmoles)
Hydroxyurea	AraCyt	Aphidicolin	ddThy	UV	
-	-	-	-	-	0.15
-	-	-	-	+	0.29
+	-	-	-	+	1.20
-	+	-	-	+	0.81
-	-	+	-	+	2.25
-	-	-	+	+	0.30
+	+	-	-	-	0.21
+	+	-	-	+	6.38
+	-	+	-	-	0.19
+	-	+	-	+	6.53
+	-	-	+	-	0.14
+	-	-	+	+	1.17

Cells at 10 hr after release from hydroxyurea block were preincubated for 1 hr with various drugs. Then cells were exposed to 50 J/m^2 UV, and incubated for another 1 hr in the presence of the same drugs as in the preincubation. Conditions for the preparation of cell lysate and dTTP[methyl-^{3}H] incorporation are as described in MATERIALS AND METHODS. Incubation time was 2 min. The concentrations of the drugs were 3 mM hydroxyurea, 10 μM araCyt, 10 μg/ml aphidicolin and 200 μM ddThy. The results are expressed as pmoles of [^{3}H]dTMP incorporated in the lysate from 1.25x10^6 cells.

araCyt did. Drug treatment alone or UV-irradiation by itself (13) did not change the profile of DNA.

DISCUSSION:

Our present results can be explained as follows: Aphidicolin inhibited the unscheduled DNA synthesis at UV-damaged sites in vivo. This inhibition was reversible and was released when the cells were washed and homogenized, and the lysate was incubated with enough amount of dXTPs. The more unscheduled DNA synthesis was inhibited in vivo, the more the incorporation of dTTP[methyl-^{3}H] was observed, because of the increase of the number of the gaps and/or the expansion of the gaps at the damaged sites.

A combined treatment of aphidicolin and hydroxyurea induced higher unscheduled DNA synthesis in vitro than aphidicolin alone. Hydroxyurea is known to reduce the level of precursors for DNA synthesis in cells by inhibiting

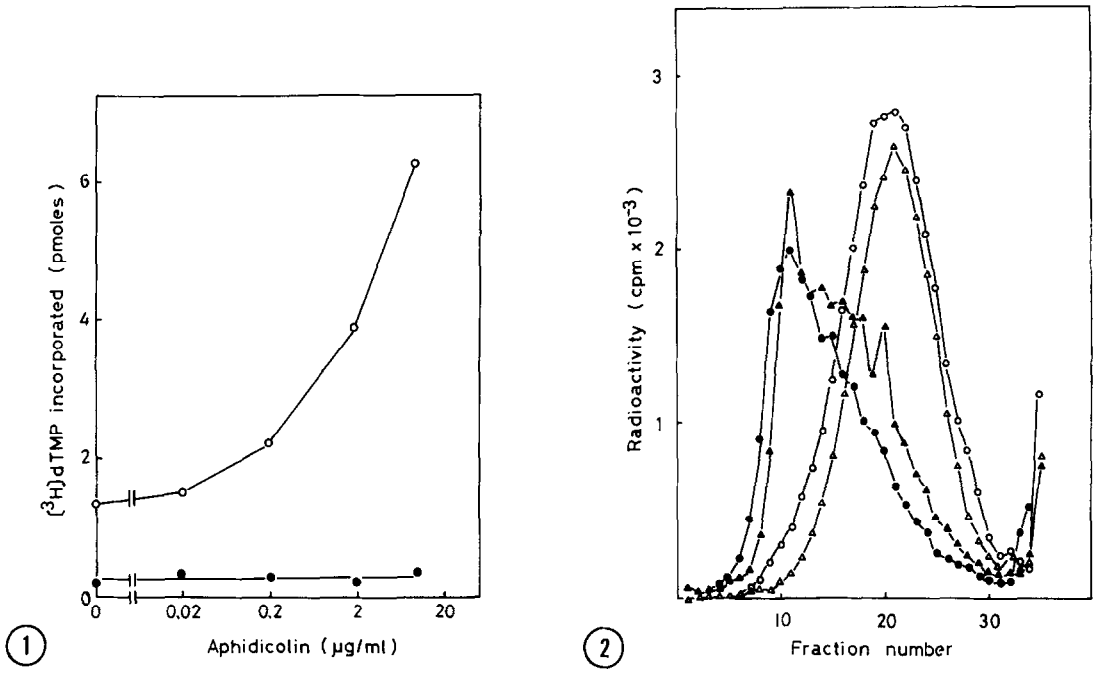

Fig. 1: Effect of aphidicolin treatment in vivo on UV-induced unscheduled DNA synthesis in HeLa cell lysate. Conditions for the experiment are as described in the legend of Table 1, except the concentration of hydroxyurea was maintained at 3 mM while the concentration of aphidicolin was varied. o, UV-irradiated; •, non-irradiated.

Fig. 2: Alkaline sucrose density gradient profiles of DNA from UV-irradiated HeLa cells. Logarithmically growing cells were incubated with thymidine[6-^{3}H] (3 μCi/ml, 5 Ci/mmol) for 20 hr. Labeled medium was replaced by regular medium for 2 hr to allow the completion of synthesis of labeled nascent DNA. The cells were incubated in the presence of 3 mM hydroxyurea plus 10 μg/ml aphidicolin or 10 μM araCyt for 1 hr, irradiated with 50 J/m^2 UV, and incubated with the same drugs for another 1 hr. Alkaline sucrose density gradient centrifugation was performed as described in MATERIALS AND METHODS.
The direction of the sedimentation was from right to left. o, UV-irradiated, hydroxyurea/araCyt-treated; •, non-irradiated, hydroxyurea/araCyt-treated; ▲, UV-irradiated, hydroxyurea/aphidicolin-treated; ▲, non-irradiated, hydroxyurea/aphidicolin-treated.

ribonucleoside diphosphate reductase (14). Considering that the inhibitory effect of aphidicolin on cell growth and DNA synthesis in vivo has been reported to be reversed by the simultaneous addition of all four deoxyribonucleosides (15), it is easy to understand why hydroxyurea and aphidicolin induced the in vitro unscheduled DNA synthesis synergistically.

We have no data at hand whether HeLa cells can convert ddThy to 2', 3'-dideoxythymidine 5'-triphosphate (ddTTP), a direct inhibitor of DNA polymerase β and γ. Abboud and Horwitz, however, observed the inhibition of Adenovirus DNA synthesis, not of nuclear DNA synthesis, in whole cells by ddThy, which is com-

parable to the results obtained by ddTTP in in vitro system (16). Thus it is reasonable to believe that ddThy was in the form that can inhibit DNA polymerases β and γ in our system. From this reason, DNA polymerase α seems to be only polymerase which is responsible for the above described unscheduled DNA synthesis.

We are still uncertain about the cause of the conflicting data concerning the roles of DNA polymerases α and β in DNA repair. Recently Miller and Chinault (17) provided the data suggesting that the extent of involvement of polymerases α and β in DNA repair synthesis is related to the amount or type of DNA damages. This needs more extensive analysis, but to us, seems plausible.

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