A Possible Involvement of DNA Topoisomerase I in “Caffeine Effect” after Ultraviolet Irradiation

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TOHDA, H., ZHAO, J.H. and OIKAWA, A. A Possible Involvement of DNA Topoisomerase I in “Caffeine Effect” after Ultraviolet Irradiation. Tohoku J. Exp. Med., 1992, 168 (2), 129–132——Caffeine has been known to enhance lethal and chromosome damaging effects of chemical and physical mutagens. In spite of numerous investigations, the mechanism is not fully elucidated. In this paper, we describe that 1) post-treatment with camptothecin (CPT), a specific inhibitor of DNA topoisomerase (topo) I, enhances SCE-induction by ultraviolet light (UV), as post-UV caffeine treatment does, 2) the lethal effect of UV is also enhanced but to a lesser degree than by post-UV caffeine treatment, and 3) caffeine, like CPT, inhibits calf thymus topo I activity, as determined by relaxation of pBR322 supercoiled DNA. These results suggest that the mechanism(s) of lethal and SCE enhancement involves the ability of caffeine to inhibit topo I. ——— caffeine; camptothecin; topoisomerase I; UV-damage repair

Recently, a role of topoisomerases in DNA repair has been suggested (Hickson et al. 1990; Smith 1990). Topo I is needed to remove a conformational stress arising from supercoiled structures of DNA during not only DNA replication but also transcription (Wang 1985). Moreover, the actively transcribed area of the genome has been known to repair UV-damage at a faster rate than non-transcribed areas (Bohr et al. 1987). Therefore, it is likely that topo I is involved in the repair process of UV-damaged DNA.

Previously, we have reported that xeroderma pigmentosum (XP) variant cells, but not normal cells, are highly sensitive to post-UV caffeine treatment in terms of sister chromatid exchange (SCE) induction (Tohda and Oikawa 1988). Here, we will describe that DNA topo I may be involved in the enhancement of SCE induction by post-UV treatment with caffeine.

MATERIALS AND METHODS

Cells and cell treatments. Cells of a human lymphoblastoid cell line, XPL19 were UV-irradiated, were grown for 3 days and viable cell number was counted. When topo inhibitors such as CPT (Sigma Chemical Co., St. Louis, MO, USA) and etoposide (a generous gift from Bristol-Myers, Squiff Co., Prinston, NJ, USA) were used, they were added to the
culture immediately after UV-irradiation and present for 3 days. The post-treatment viability was calculated by dividing the viable cell number in irradiated culture by that in control culture as stated by Moshell et al. (1981).

**Measurement of SCE.** Cells were irradiated and allowed to grow in BrdUrd (10 μM)-containing medium in the presence or absence of CPT. Differential staining of sister chromatids was carried out by fluorescence plus Giemsa technique (Perry and Wolff 1974).

**Topoisomerase assay.** Topo I activity was measured by relaxation of superhelical plasmid DNA. pBR322 DNA was incubated with calf thymus topo I (BRL) in the 20 μl of assay mixture containing 50 mM Tris-HCl (pH 7.8), 120 mM KCl, 10 mM MgCl₂, 0.5 mM EDTA, 0.5 mM dithiothreitol and 30 μg/ml BSA at 37° for 30 min. The reactions were terminated by the addition of stop solution and analyzed in 1.2% agarose gels (Liu and Miller 1981).

**RESULTS AND DISCUSSION**

Effects of topo inhibitors on UV-induced SCEs and cell killing were examined. As shown in Fig. 1, CPT induced SCEs at low concentrations of 0.1 and 0.2 nM. When CPT was added to UV-irradiated cell culture, the levels of SCE were consistently higher than expected for simple additive effect of UV and CPT. Etoposide, an inhibitor of topo II, did not show any synergistic enhancement at the concentration of 1 nM (Fig. 1B), suggesting that inhibition of topo I, but not topo II results in the SCE enhancement.

To examine the effect of CPT on the killing effect of UV, irradiated or non-irradiated cells were incubated for 3 days in the presence or absence of CPT. As shown in Fig. 2, the viable cell ratios at 3 days after UV decreased dose dependently. CPT itself did not change the growth ratio at 0.1 nM but reduced it slightly at 0.5 nM. Combination treatment with UV and CPT resulted in a synergistic reduction in the viable cell ratio, particularly at a higher dose of 10 J/m². The results suggest that a post-treatment with CPT enhances not only...
UV-induced SCEs but also UV-killing. The similar enhancing effect of caffeine has been observed in UV-irradiated cells (Tohda and Oikawa 1983, 1988).

Since CPT is a specific inhibitor to topo I, we examined the influence of caffeine on calf thymus topo I. As seen in Fig. 3, caffeine inhibited dose dependently the relaxation of supercoiled DNA produced by topo I. DNA intercalators such as adriamycin (ADM) can change the mobility in the gel only by virtue of the binding to DNA. According to Tornaletti et al. (1989), caffeine can also intercalate to DNA. However, our results show that caffeine did not change the supercoiled structure in the absence of topo I (Fig. 3, lanes 2 & 3). Moreover, when relaxed pBR322 DNA was used as a substrate, ADM changed electrophoretic mobility but caffeine did not (data not shown). These results suggest that caffeine acts via inhibition of topo I, because of very weak ability to intercalate to DNA, if any.

Fig. 2. Effect of CPT on UV-induced cell killing
■, without CPT; □, 0.1 nM CPT; △, 0.5 nM CPT.

Fig. 3. Inhibitory effect of caffeine on topo I activity.
Recently, post-treatment with $\beta$-lapachone, an activator of topo I, was found to enhance cell killing by x-ray (Boothman et al. 1989), suggesting that activation of topo I interferes with the repair of damaged DNA. Our results, together with their results, indicate that modification of topo I activity, either activation or inhibition, interferes with repair of DNA damage. Caffeine may result in a conformational change in DNA via modification of topo I activity. In UV-irradiated cells, such changes may inhibit repair process, leading to SCE enhancement and increased cell killing.

References


