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INDUCTION OF DIFFERENTIATION OF HUMAN AND MOUSE MYELOID LEUKEMIA CELLS BY CAMPTOTHECIN

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SUMMARY: Low concentrations of camptothecin induced differentiation of human and mouse myeloid leukemia cells including human HL60, U937, ML1, and K562 cells and mouse M1 cells as measured by various differentiation-associated properties. When K562 cells were pretreated with 20 nM camptothecin for 2 h, 53% of the cells were induced to differentiate as measured by NBT staining. Significant single strand breaks in DNA of K562 cells were caused by this treatment. Most single strand breaks were accompanied by protein-DNA cross linking. The combination of camptothecin and rTNF synergistically induced differentiation of human ML1, U937, and M1 cells. These results suggest that topo I may be important in some differentiation of myeloid leukemia cells.

Increasing attention has been focused on differentiation therapy of leukemia using differentiation inducing compounds and cytokines. For this purpose, various compounds and cytokines with strong differentiation inducing activity for leukemia cells and their combinations to obtain the strongest possible differentiation inducing activity have been investigated (1-3). In the present study, we examined whether camptothecin, an inhibitor of topo I, may induce differentiation in human and mouse myeloid leukemia cells. The first report suggesting the

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The abbreviations used are: NBT, nitroblue tetrazolium; topo I, DNA topoisomerase I; topo II, DNA topoisomerase II.

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involvement of DNA topoisomerase in the differentiation of leukemia cells was made by Bodley, Wo, and Liu (4), who showed that topo II activity of erythroleukemia cells was decreased significantly during differentiation. Recently, Constantinou, Henning-Chubb, and Huberman (5) demonstrated that human leukemia HL60 cells were induced to differentiate by novobiocin, an inhibitor of topo II, and that the induction was associated with reduction in topo II activity. This is the first report demonstrating that differentiation of various myeloid leukemia cells was induced by topo I inhibitor.

MATERIALS AND METHODS

Materials Camptothecin was kindly provided by Dr. M. Fukuda of Aichi Medical Univ. Human rTNFα (1.1×10^8 U/mg) was supplied by Fujisawa Pharmaceutical Co. Mouse leukemia M1 cells, Human leukemia HL60, ML1, and U937 cells were grown as described previously (3,6,7). Human leukemia K562 cells were kindly supplied by Dr. K. Takeda of Showa University and cultured in RPMI1640 medium (Flow Laboratories, Inc.) supplemented with 10% (v/v) fetal calf serum at 37°C under 5% CO_2 in air.

Assay for differentiation inducing activity Leukemia cells (1×10^5 cells/ml) were treated for 3 days with sample solutions containing various concentrations of camptothecin. NBT reducing ability of the cells was measured by incubating the cells with NBT for 20 min at 37°C, and then examining with a microscope. Phagocytic activity was determined by measuring the capacity of the cells to engulf polystyrene latex particles (average diameter, 0.81 μm; Difco Laboratories). The cytochemical stains for α-naphthyl acetate esterase and AS-D chloroacetate esterase were examined in smear preparations as described previously (3,6,7). Appearance of Fc receptors was assayed by measuring erythrocyte-antibody rosette formation, using sheep erythrocytes coated with rabbit antisheep erythrocyte antibodies (8).

Alkaline elution The number of alkali-labile sites plus complete single strand break in DNA was analyzed by the alkaline elution technique of Kohn, Ewig, and Erickson (9) as described previously (10). Briefly, the cells, after treatment with camptothecin, were lysed on polycarbonate filters (25 mm diameter, Nuclepore Corp, Pleasanton, CA) with proteinase K (0.5 mg/ml) in the presence of 2% sodium dodecyl sulfate, then washed with 20 mM EDTA (pH 9.6) and eluted with 50 mM tetrapropylammonium hydroxide (pH 11.9) at a rate of 0.05 ml/min. Fractions were collected directly in scintillation vials at 90 min intervals. Radioactivity remaining on the filter was recovered by heating the filter at 70°C for 1 h in 0.5 ml of 1 N HCl, followed by addition of 0.5 ml of 1 N NaOH. As a standard for DNA-strand breaks, cells were irradiated with 300 rad of γ rays from ^{60}Co (the dose rate was 30 R/min) at 0°C and harvested. The amount of protein-DNA cross-linking was analyzed by lysing the cells with proteinase K as described above, and also without proteinase K in the presence of 0.2% Sarkosyl (sodium lauryl sarkosinate) as described previously (11) and the difference in

the number of single strand breaks calculated from the elution profiles obtained from either treatment was attributed to protein-DNA cross-linking.

RESULTS

Differentiation of leukemia cells by camptothecin

Table I shows effects of camptothecin, an inhibitor of Topo I, on various characteristics of differentiation markers of human and mouse myeloid leukemia cell lines. The concentrations of camptothecin were chosen below the concentrations in which cell viability did not decrease less than 90% as determined by Trypan blue staining. Of the M1 cells treated with 90 nM camptothecin, 51% were stained with α-napthyl acetate esterase stain. This indicated that the cells were differentiated into monocytic

Table 1. Induction of differentiation markers and growth inhibition in various myeloid leukemia cells

Cell line	Concentration (nM)	Inhibition (%)	Cell exhibiting (%)				
			NBT reduction	ASD-Chloroacetate activity	α-Naphthyl acetate activity	Fc-receptor	Phagocytosis
K562	20	81 ± 3	88 ± 11 (< 1)	90 ± 3 (< 5)	< 5 (< 1)	30 ± 1 (< 3)	31 ± 12 (< 5)
HL60	3	40 ± 4	5 ± 3 (< 4)	< 5 (< 1)	40 ± 4 (< 3)	32 ± 5 (< 1)	47 ± 2 (< 4)
ML1	3	50 ± 6	5 ± 3 (< 2)	53 ± 6 (8±6)	< 4 (< 1)	52 ± 5 (13±3)	61 ± 6 (< 4)
U937	3	23 ± 2	< 5 (< 1)	14 ± 1 (< 5)	< 6 (< 3)	31 ± 2 (7±5)	16 ± 4 (9±4)
M1	90	40 ± 1	17 ± 1 (< 1)	20 ± 6 (< 1)	51 ± 3 (< 1)	< 6 (< 1)	19 ± 9 (< 1)

K562, HL60, ML1 and U937 cells were incubated with camptothecin at the indicated concentrations for 4 days at 37 C. M1 cells were treated the same, but for 3 days. The percentages are the mean ± SD from at least four experiments. Figures in parentheses represent values obtained for untreated cells.

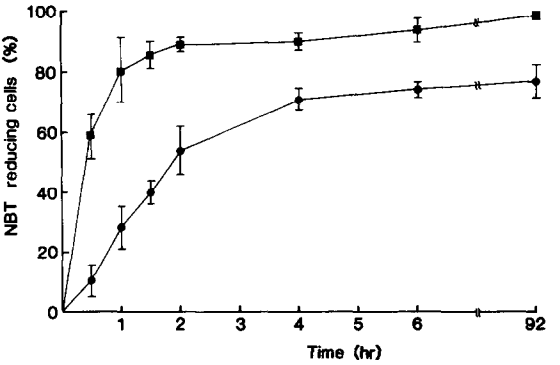

Fig. 1. Effects of pretreatment with camptothecin on differentiation of leukemia cells. K562 cells were pretreated with 20 nM (●) and 700 nM (■) camptothecin for the indicated times at 37°C. After washing the medium by centrifugation, the cells were incubated in the culture medium without camptothecin for 3 days. Differentiation-inducing activity of camptothecin was measured by NBT staining as described in the text. Each value is mean ± S.D. of triplicate determinations.

cells. HL60 cells treated with 3 nM camptothecin were also highly stained by α-napthyl acetate esterase. In contrast, ASD-chloroacetate esterase activity of ML1 cells and K562 cells treated with camptothecin increased markedly, indicating that these cells were differentiated into granulocytic cells. Ttreatment of U937 cells with 3 nM camptothecin increased α-naphthyl acetate esterase activity and ASD-chloroacetate esterase activity only slightly, but the number of Fc-receptors and phagocytosis activity were significantly increased by this treatment.

Time dependence of pretreatment with camptothecin

Fig. 1 shows effects of pretreatment with various concentrations of camptothecin on the differentiation of K562 cells as measured by NBT staining. After pretreatment of the cells with camptothecin, the reagent was removed by washing, and the cells were further incubated for 3 days. After pretreatment with 20 nM or 700 nM camptothecin for 2 h, 53% and 89%, respectively, of the cells became stainable with NBT, indicating that camptothecin induced irreversible change in the cells within a few hours.

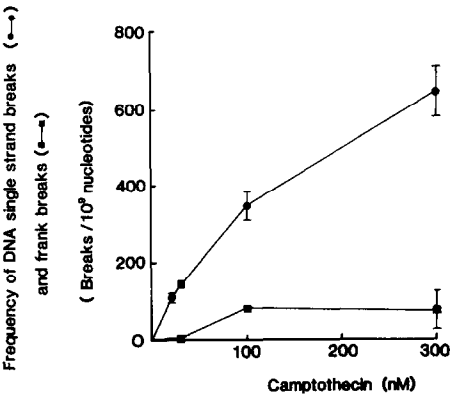

Fig. 2. Frequency of DNA single strand break in K562 cells as a function of camptothecin concentrations. K562 cells were treated with camptothecin for 2 hrs at 37°C. After treatment with camptothecin, cells were lysed with proteinase K in the presence of 2% sodium dodecyl sulfate (●) or without proteinase K in the presence of 0.2% Sarkosyl (■), followed by the alkaline elution. Each value is mean ± S.D. of duplicate determinations.

Effect on DNA single strand break

Camptothecin is known to interfere with the breakage-reunion reaction of topo I by stabilizing a cleavable complex between topo I and DNA (9). Fig. 2 shows the effects of camptothecin on a DNA single strand break in K562 cells. After treatment with 20 nM of camptothecin for 2 h, about 100 single strand breaks per 10^9 dalton DNA were produced. To check whether the DNA single strand breaks produced by treatment with camptothecin were the result of protein-DNA cross linking, K562 cells were lysed without proteinase K in the presence of 0.2% Sarkosyl. The number of single strand breaks without proteinase K digestion was less than 24% of that with proteinase K digestion at the camptothecin concentrations examined. That is to say, almost 80% of single strand breaks were accompanied by protein-DNA cross-linking. These results are consistent with the interpretation that camptothecin stabilized a cleavage complex between topo I and DNA even in K562 cells.

Synergistic effects of camptothecin and rTNF

Fig. 3 shows the synergistic effects of camptothecin plus rTNF. In this experiment, the concentrations of camptothecin and

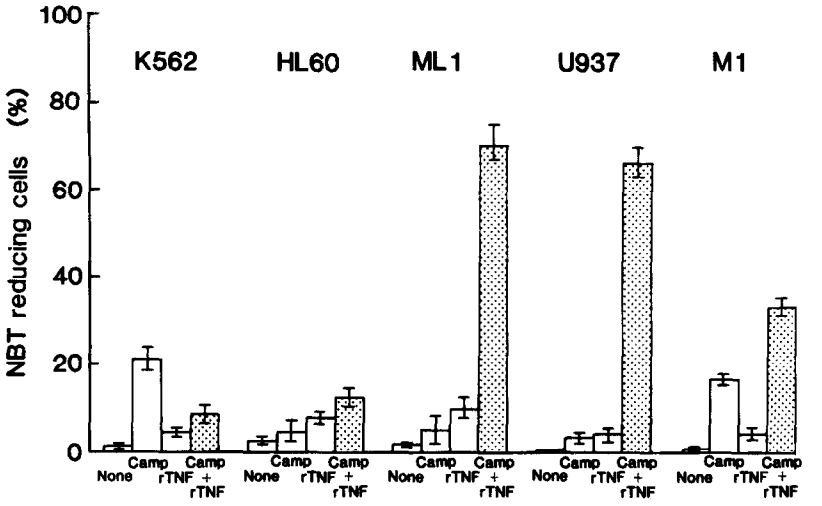

Fig. 3. Synergistic effect of topo I inhibitor and rTNF on the differentiation of leukemia cells. Leukemia cells were treated for 3 days with camptothecin alone or in combination with rTNF. Differentiation inducing activity of camptothecin or its combination with rTNF was measured by NBT staining as described in the text. The concentration of rTNF used was 3.4×10^5 U/ml for K562, HL60, ML1, U937 cells and 1.7×10^5 nM for M1 cells. The concentration of camptothecin used is 10 nM for K562 cells, 90 nM for M1 cells and 3 nM for HL60, ML1, and U937 cells. Each value is mean $\pm$ S.D. of triplicate determinations. Camp is the abbreviation of camptothecin.

rTNF were decreased to show the synergistic effects more clearly. Remarkable synergis was observed when U937, ML1, and M1 cells were treated with camptothecin plus rTNF. No synergistic effect of camptothecin plus rTNF was observed for K562 or HL60 cells.

DISCUSSION

Myeloid leukemia cells can be induced to differentiate by various cytokines such as rTNF (6,12,13) and IFN-γ (14,15); low molecular weight compounds including 1α,25 dihydroxyvitamin D_3 (16), retinoic acid (17,18); and antileukemic drugs such as 1-β-D-arabinofuranosylcytosine and actinomycin D (2). The present study clearly demonstrates that camptothecin induces differentiation in all five myeloid leukemia cell lines examined. We interpreted that the inhibition of topo I by camptothecin could be a trigger of the differentiation of these cells for the following reasons: 1) The concentration of camptothecin effective

for inducing differentiation is so low that cell viability was not practically changed (less than 10% decrease). 2) Incubation of K562 cells with 20 nM camptothecin for 2 hrs is enough to induce differentiation in more than 50% of the cells. This treatment produced significant single strand breaks of DNA in K562 cells.

Constantinou, Henning-Chubb, and Huberman (5) reported that camptothecin had no differentiation-inducing activity in HL60 cells, whereas we observed, in the present study, that differentiation is induced by camptothecin even in HL60 cells. A simple explanation for this discrepancy is that additional compounds other than camptothecin such as cytokines present in the serum or in leukemia cells may contribute to the induction of differentiation in leukemia cells by camptothecin. We showed, in the present study, that differentiation by camptothecin is synergistically increased by rTNF. Synergistic effects of rTNF with other compounds in differentiation of various leukemia cells have been reported (3,12,13,19,20). Elucidation of the mechanism of induction of differentiation by camptothecin might lead to explore a new reagent suitable for differentiation therapy for leukemia.

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