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Letter to the Editor

The influence of G_2 arrest abrogation on the long-term cytotoxicity of different genotoxic lesions

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The ability of DNA-damaged cells to arrest in the G_2 phase of the cell cycle is believed to enhance cellular survival by providing additional time for the repair of DNA lesions.[1,2] Arrest in G_2 is regulated by checkpoint control systems and can be abrogated by chemical inhibition of checkpoint kinases.[3] Combined treatment of tumor cells with conventional DNA-damaging agents and G_2 checkpoint abrogators often leads to enhanced cytotoxicity and improved therapeutic effects.[4,5] The main goal of the current work was to establish the impact of G_2 arrest, transition into mitosis and induction of apoptotic cell death on the long-term viability of tumor cells following exposure to genotoxic agents. Specifically, we investigated the influence of different classes of DNA damaging agents on the cell cycle progression and induction of cell death after exposure to sub-lethal doses (IC_{90} concentrations) according to the scheme outlined in Figure 1A. To this end, we used two different cellular systems, human T cell leukemia MOLT-4 and murine myeloid leukemia M1 cells. Exposure of both cell types to DNA alkylators or topoisomerase poisons was accompanied by induction of growth inhibition and cell cycle arrest in G_2 (Fig. 1B upper and C). Western blot analysis of the phosphorylation status of the major mitotic kinase, Cdk1 kinase, was carried out to establish that the G_2/M arrested cells were arrested in G_2 rather than in M. Experimental data, obtained for both camptothecin- or etoposide-treated cells, show that growth arrest induced by these drugs was accompanied by the appearance of a band with lower electrophoretic mobility in SDS-PAGE gels, indicative of the inactive, phosphorylated form of Cdk1. This was observed for both MOLT-4 (Fig. 1B lower) and for M1 cells (Fig. 1C lower). Moreover, the MPM-2 mitotic phospho-epitope was not detectable for either MOLT-4 (Fig. 1B lower) or M1 (Fig. 1C lower). This is in agreement with our previous findings with melphalan[6] and cisplatin.[7] To determine the influence of the camptothecin- or etoposide-induced G_2 arrest on cell survival, a non-toxic dose of the cell cycle modulators, caffeine (1 mM) or UCN-01 (100 nM), were added to the growth arrested cells. The addition of caffeine or UCN-01 resulted in a rapid G_2 exit and mitotic entry as revealed by the appearance of the MPM-2 phosphoepitope for both MOLT-4 (Fig. 1B lower) and M1 cells (Fig. 1C lower). Similar changes of the MPM-2 staining were observed by biparametric flow cytometry analysis of the two cell lines (data not shown). Moreover, G_2 exit was accompanied by a gradual activation of Cdk1 kinase, as shown by the conversion of the inactive, phosphorylated form of Cdk1 into the dephosphorylated, active form of the kinase in MOLT-4 (Fig. 1B lower) and M1 (Fig. 1C lower). Taken together these results, in combination with previous findings,[6,7] indicate that treatment of MOLT-4 and M1 cells with different classes of DNA damaging agents including cisplatin, melphalan, camptothecin and etoposide led to induction of G_2 arrest which, in all cases, was sensitive to both caffeine and UCN-01. No measurable caspase-3 activity, one of the major execution caspases, was detected in the growth-arrested MOLT4 cells while a marginal induction of cell death was observed. In contrast, post-incubation of G_2-arrested cells in the presence of caffeine was associated with a clear induction of caspase-3 measured by catalytic activity assays (Fig. 2A left) as well as by biparametric flow cytometry analysis detecting the cleaved, active form of the enzyme (Fig. 2A right). A comparable activation of caspase 3 was also observed for M1 cells treated with cisplatin,[7] camptothecin or etoposide (this study, data not shown) after post-incubation with UCN-01. Next, DNA fragmentation was used as an independent marker of apoptotic cell death. Post-incubation of G_2-arrested M1 cells with UCN-01 led to enhanced DNA fragmentation for all DNA damaging agents studied[7] (and data not shown). In parallel, microscopic examination of the nuclear morphology of camptothecin- or etoposide-treated M1 cells confirmed the accelerated DNA fragmentation of G_2-arrested cells in the presence of UCN-01 (Fig. 2B). Internucleosomal DNA fragmentation is one of the most specific markers of apoptotic cell death and is characteristic of the advanced stages of apoptosis. While no internucleosomal DNA fragmentation was observed in control or G_2-arrested MOLT-4 cells, caffeine induced rapid DNA fragmentation in parallel with G_2 to M transition (Fig. 2C). The internucleosomal fragmentation observed after drug-treatment is indicative of an important induction of apoptosis, since MOLT-4 cells contain low levels of nuclear endonuclease activity.[8] These results demonstrate, that for all drugs studied, post-incubation of G_2-arrested cells with caffeine or UCN-01 resulted in G_2 arrest abrogation and induction of apoptotic cell death. We next wanted to establish whether G_2 arrest abrogation with caffeine- or UCN-01 was accompanied by an increased long-term cytotoxicity. Cells were treated with either drug for the time required to establish G_2 arrest (18 hr for MOLT-4 and 24 hr for M1), post-incubated in the absence or presence of caffeine or UCN-01 for 8 and 3 hr, respectively, and further post-incubated in drug-free media. Unexpectedly, G_2 arrest override was not associated with increased long-term cytotoxicity of etoposide or camptothecin in MOLT-4 cells (Fig. 2D, upper). In marked contrast, post-incubation with caffeine dramatically increased the cytotoxicity of melphalan toward the same cell type (Fig. 2D, upper). Similarly, UCN-01 significantly sensitized M1 cells to melphalan (Fig. 2D, lower) but had no detectable influence on the long-term viability of campthotehcin-treated cells (Fig. 2D, lower). Etoposide-treatment represented an intermediary situation, since checkpoint override influenced the long-term viability at low, but not at high drug-concentrations (Fig. 2D, lower). Therefore, although caffeine and

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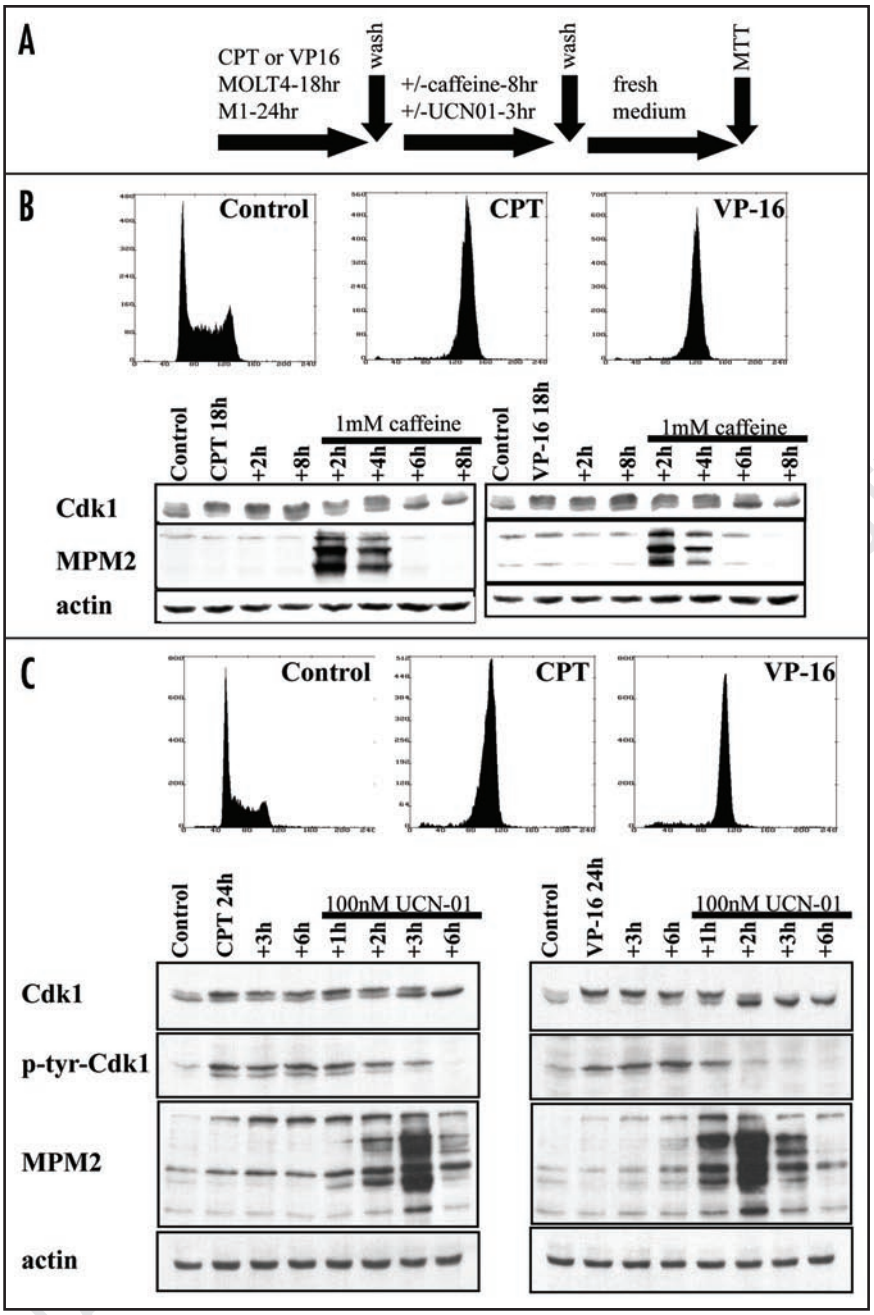

Figure 1. (A) Drug scheduling of DNA-damaging agents and cell cycle abrogators used in this study. (B) DNA histograms of MOLT-4 cells treated with camptothecin or etoposide for 18 hr as determined by flow cytometry (upper panel). Cdk1 phosphorylation and expression of the MPM-2 phosphoepitope in MOLT-4 cells treated with camptothecin or etoposide for 18 hr followed by postincubation in the abseince or presence of caffeine as determined by Western blot analysis (lower panel). (C) DNA histograms of M1 cells treated with camptothecine or etoposide for 24 hr as determined by flow cytometry (upper panel). Cdk1 phosphorylation and expression of the MPM-2 phosphoepitope in M1 cells treated with camptothecin or etoposide for 24 hr followed by postincubation in the presence or absence of UCN-01 as determined by Western blot analysis (lower panel).

UCN-01 were both able of G_2 checkpoint override and rapid induction of apoptotic cell death in cells treated with different classes of DNA damaging agents, check-point override was accompanied by increased long-term cytotoxicity only for DNA crosslinking agents (melphalan and cisplatin) but not for topoisomerase inhibitors (etoposide and camptothecin). Many tumor cell lines are deficient for one or several DNA repair pathways. Therefore, it is possible that the lack of repair during etoposide- or camphothecin-induced G_2 arrest might be caused by deficiency in the appropriate repair pathways. However, this possibility seems unlikely since comparable results were obtained for two non-related cell lines of different origins (MOLT-4 and M1). Moreover, similar findings have been reported for RKO colorectal tumor cells.[9] Alternatively, the nature of DNA lesions produced by alkylating agents and by topoisomerase inhibitors may be different, and it is possible that the G_2 arrested cells are not able to repair the DNA damage which resulted from topoisomerase inhibition. Interestingly, we consistently observed a shoulder for the cytotoxicity curve for low doses of etoposide (corresponding to about IC_{10}–IC_{20} concentration) in combination with caffeine or UCN-01 (see Fig. 2D). This may suggest that low levels of etoposide-induced DNA damage are repairable during G_2 phase for

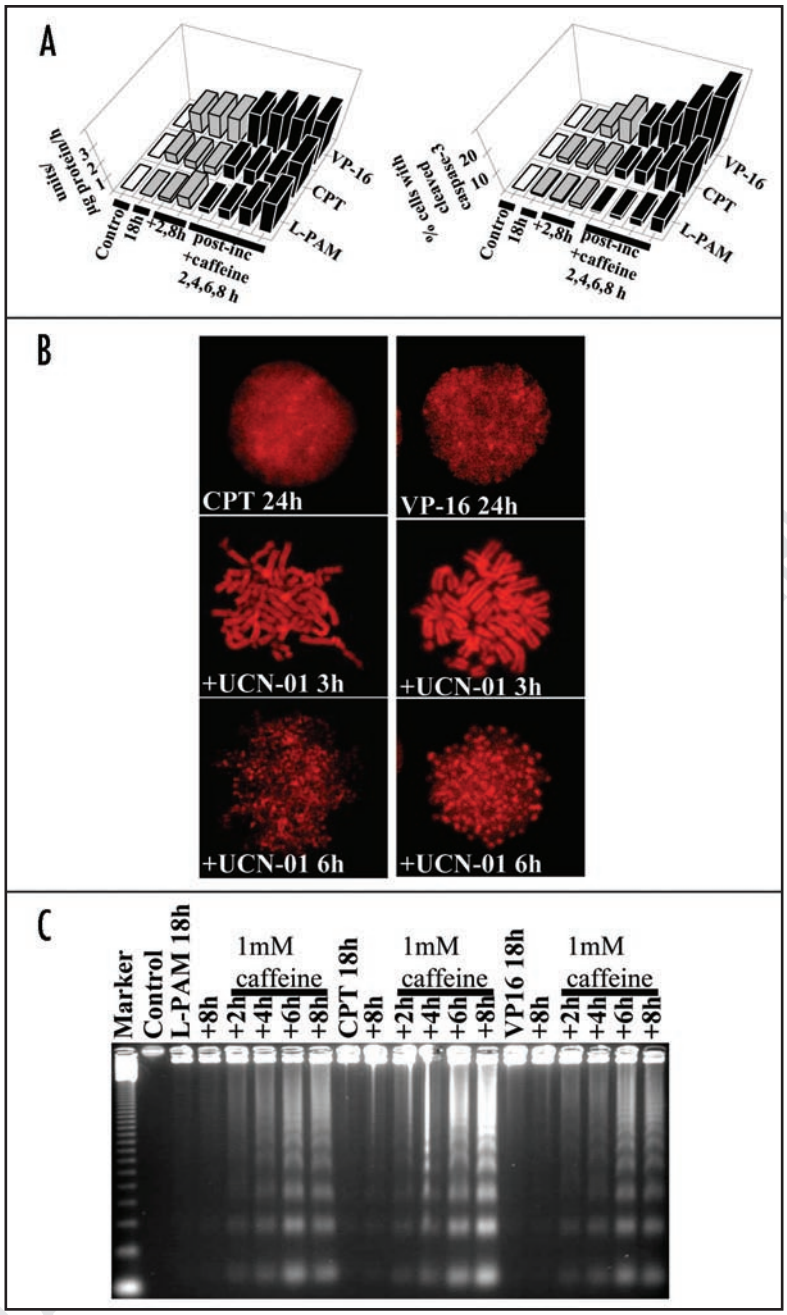

Figure 2. (A) Activation of caspase-3 in MOLT-4 cells treated with melphalan, camptothecin or etoposide for 18 h followed by post-incubation in the absence or presence of caffeine as determined by an enzymatic assay (left) or flow cytometry of the cleaved active form of the enzyme (right). (B) Influence of UCN-01 on chromatin morphology in M1 cells exposed to camptothecin (left) or etoposide (right). (C) Analysis of internucleosomal DNA fragmentation in MOLT-4 cells treated with melphalan, camptothecin or etoposide for 18 h followed by post-incubation in the absence or presence of 1 mM caffeine. (D) Long term viability as determined by the MTT assay. MOLT-4 cells were treated with melphalan, camptothecin or etoposide for 18 hr followed by post-incubation in the absence (○) or presence (●) of caffeine for 8 hr and further incubation in drug-free media for 48 h (upper). Alternatively, M1 cells were exposed to melphalan, camptothecine or etoposide for 24 hr followed by post-incubation in the absence (○) or presence (●) of UCN-01 for 3 hr and further incubation in drug-free media for 96 hr (lower panel).

which reason abrogation of the DNA damage checkpoint by caffeine and UCN-1 is able to enhance the long-term cytotoxic effects. Together, our results clearly demonstrate that abrogation of G_2 arrest in cells treated with genotoxic agents is not necessarily associated with long-term cell survival. Therefore, the therapeutic benefit from the use of cell cycle abrogators, which modulate DNA damage checkpoint, depends on the nature of DNA lesions induced by cytotoxic agents.

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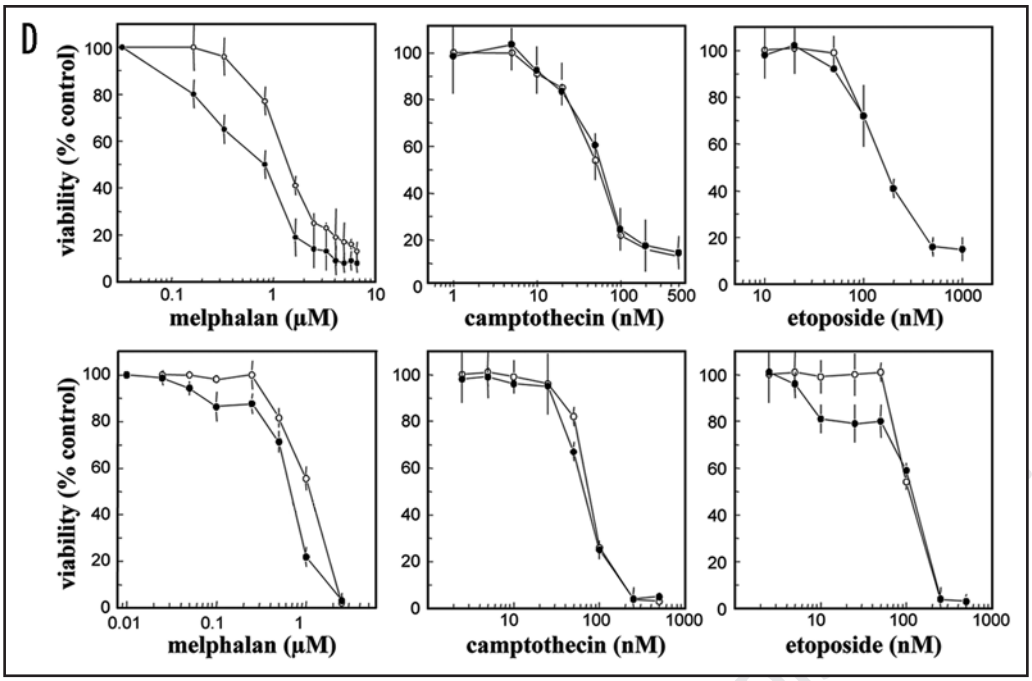

Figure 2. (D) For legend, see page 3.

References

1. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science 1989; 246:629-34.
2. Goldwasser F, Shimizu T, Jackman J, Hoki Y, O'Connor PM, Kohn KW, Pommier, Y. Correlations between S and G_2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. Cancer Res 1996; 56:4430-7.
3. Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA and O'Connor PM. UCN-01: a potent abrogator of G_2 checkpoint function in cancer cells with disrupted p53. J Natl Cancer Inst 1996; 88:956-65.
4. Eastman A. Cell cycle checkpoints and their impact on anticancer therapeutic strategies. J Cell Biochem 2004; 91:223-31.
5. Tenzer A, Pruschy M. Potentiation of DNA-damage-induced cytotoxicity by G_2 checkpoint abrogators. Curr Med Chem Anticancer Agents 2003; 1:35-46.
6. Bozko P, Sabisz M, Larsen AK and Skladanowski A. Cross-talk between DNA damage and cell survival checkpoints during G_2 and mitosis: pharmacological implications. Molecular Cancer Therapeutics 2005; 4:2016-25.
7. Skladanowski A, Bozko P, Sabisz M and Larsen AK. Dual inhibition of PI3K/Akt signaling and the DNA damage checkpoint in p53-deficient cells with strong survival signaling: implications for cancer therapy. Cell Cycle 2007; 6:2268-75.
8. Beere HM, Chresta CM, Alejo-Herberg A, Skladanowski A, Dive C, Larsen AK, Hickman JA. Investigation of the mechanism of higher order chromatin fragmentation observed in drug-induced apoptosis. Mol Pharmacol 1995; 5:986-96.
9. Bozko P, Larsen AK, Raymond E, Skladanowski A. Influence of G_2 arrest on the cytotoxicity of topoisomerase inhibitors toward human carcinoma cells with different p53 status. Acta Biochimica Polonica 2002; 49:109-19.