Cytoxic signalling by inhibitors of DNA topoisomerase II

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Abstract

DNA topoisomerase (topo) II inhibitors either stabilize DNA–topo II complexes by blocking DNA religation (e.g. etoposide) or block the enzyme’s catalytic activity (e.g. dexrazoxane). The former class of drugs causes direct DNA damage through topo II, while the latter class does not, but both classes cause apoptosis. We cloned the Fas ligand (FasL) promoter and coupled it to the luciferase gene. Treatment of cells transfected with this construct revealed that complex-stabilizing (DNA-damaging) agents induce FasL expression, but the catalytic inhibitors do not, suggesting that the FasL pathway may not be involved in all cases of topoisomerase-mediated apoptosis. Some topo II inhibitors activate a pathway involving stress-activated protein kinases, which include c-Jun N-terminal kinase-1 (JNK-1). We will discuss the effects of these agents on components of this pathway. Our earlier work revealed that topo IIα interacts with the cell cycle regulatory protein, retinoblastoma protein (Rb). This interaction and the subcellular distribution of these proteins are altered by topo II inhibitory drugs and lead to apoptosis. In addition, agents that affect Rb, such as E1A and E2F1/DP-1, when transfected into cells, also alter topo IIα–Rb localization, activate jun kinase pathways and cause apoptosis. This paper discusses current studies that are designed to determine the contributions of these signalling events to the alterations in subcellular protein distribution and apoptosis. We suggest that protein–protein interactions are important for mediation of cytoxic signalling by anticancer drugs.

DNA topoisomerase (topo) enzymes are important nuclear enzymes that regulate DNA metabolism and affect replication, transcription, recombination, chromatin assembly, possibly DNA repair and, ultimately, cell division. There are two classes of topoisomerases in mammalian cells: the type I enzymes, which cut and pass single strands of DNA, and the type II enzymes, which cut and pass double-stranded DNA. Five topo enzymes in these classes have been identified in mammalian cells, but the three that have significant consequences for cancer and cancer chemotherapy are topo I, topo IIα and topo IIβ [1]. Indeed, important chemotherapeutic agents that are in clinical use or are the subjects of clinical trials target these enzymes. These include etoposide and doxorubicin (topo II inhibitors) and topotecan and irinotecan (topo I inhibitors).

Inhibitors of topo II either stabilize DNA–topo II complexes by blocking DNA religation and causing direct DNA damage (e.g. etoposide) or by blocking the enzyme’s catalytic activity (e.g. dexrazoxane) without causing DNA damage [1].

It has been known for some time that DNA damaging, topo II inhibitory anticancer drugs, such as the epipodophyllotoxins (etoposide) and anthracyclines (doxorubicin), cause apoptosis. Less clear was whether the catalytic inhibitors could also promote apoptosis. We showed that the catalytic inhibitors of topo II (e.g. merbarone, dexrazoxane (ICRF-187; (S)-4,4’-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione) also caused apoptosis in human leukaemic cell lines [2,3]. However, the mechanism for the induction of apoptosis is less well understood.

Key words: catalytic inhibitor; jun kinase signalling.
Abbreviations used: FasI, Fas ligand; GFP, green fluorescent protein; JNK-1, c-Jun N-terminal kinase-1; topo, topoisomerase.

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It has been shown that etoposide and doxorubicin activate c-Jun N-terminal kinase-1 (JNK-1) and its downstream target, c-Jun [4,5]. Activation of this stress-activated protein kinase pathway is required for the induction of apoptosis by such drugs [6]. This subject has been comprehensively reviewed recently [6].

We will discuss our new results demonstrating that MEKK4, which activates JNK-1, is also activated (phosphorylated) by treatment of cells with topo II inhibitors (U. G. Bhat et al., unpublished data). JNK-1 activation in turn, not only activates and phosphorylates c-Jun as discussed above, but it also activates (phosphorylates) activating transcription factor 2. We have found also that topo II inhibitors activate activating transcription factor (U. G. Bhat et al., unpublished data).

We investigated whether upstream events were involved in such cytotoxic signalling by these topo inhibitors, and focused our early efforts on activation of the Fas ligand (FasL). We cloned the FasL promoter and coupled it to the luciferase gene [7]. Treatment of cells transfected with this construct revealed that complex-stabilizing (DNA-damaging) agents induce FasL expression, but the catalytic inhibitors do not [7]. However, in both cases, these different classes of drugs cause apoptosis, suggesting that the FasL pathway may not be involved in all cases of topo-mediated apoptosis.

Since both classes of inhibitors activate a pathway involving JNK-1, whether they damage DNA or not, this suggested that their cytotoxic actions may involve some other cellular functions or proteins that interfere with cell-cycle regulation. Accordingly, we decided to focus our efforts on downstream events. We had shown earlier that topo IIα interacts physically and functionally with the cell cycle regulatory protein, Rb, suggesting novel regulatory mechanisms for both proteins [8]. To explore this phenomenon in greater detail, we used our previously-described green fluorescent protein (GFP)-topo IIα fusion proteins [9,10], which are catalytically active. We transfected HeLa cells with GFP-topo IIα and used fluorescence microscopy to examine the nuclear distribution of this protein. We also used Rb-specific antibodies and immunohistochemistry to examine its subcellular distribution. Our results suggest that these proteins co-localize in the nucleus. Moreover, we investigated whether these interactions can be visualized in vivo using fluorescence methods and whether drugs or proteins can affect the interactions. Our results suggest that the interaction between topo IIα and Rb and the subcellular distribution of these proteins are altered by topo II inhibitory drugs, which leads to apoptosis. In addition, agents that affect Rb, such as E1A and E2F1/DP-1, when transfected into cells, activate jun kinase pathways and cause apoptosis [11,12]. We found that under these conditions, these transfected Rb-interacting proteins also alter topo IIα–Rb localization (U. G. Bhat et al., unpublished data).

Overall, our results suggest that disruption of the topo IIα–Rb interaction by drugs that interfere with topo II or by proteins that interfere with Rb can alter the nuclear distribution of these topo II and Rb proteins and can induce apoptosis. We suggest that protein–protein interactions are important for mediation of cytotoxic signalling by anticancer drugs. Studies are presently underway to determine the contributions of these signalling events to the alterations in subcellular protein distribution and apoptosis.

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References


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