Apoptotic pathway induced by noscapine in human myelogenous leukemic cells

Nastaran Heidari[a], Bahram Goliaei[a], Parvaneh Rahimi Moghaddam[b,c], Nahid Rahbar-Roshandel[b,c] and Massoud Mahmoudian[b,c]

It has been shown that noscapine, an opium-derived phthalideisoquinoline alkaloid that is currently being used as an oral antitussive drug, induces apoptosis in myeloid leukemia cells. The molecular mechanism responsible for the anticancer effects of noscapine is poorly understood. In the current study, the apoptotic effects of noscapine on two myeloid cell lines, apoptosis-proficient HL60 cells and apoptosis-resistant K562 cells, were analyzed. An increase in the activity of caspase-2, -3, -6, -8 and -9, poly(ADP ribose) polymerase cleavage, detection of phosphatidylserine on the outer layer of the cell membrane, nucleation of chromatin, and DNA fragmentation suggested the induction of apoptosis. Noscapine increased the Bax/Bcl-2 ratio with a significant decrease of Bcl-2 expression accompanied with Bcl-2 phosphorylation. Using an inhibitory approach, the activation of the caspase cascade involved in the noscapine-induced apoptosis was analyzed. We observed no inhibitory effect of the caspase-8 inhibitor on caspase-9 activity. In view of these results and taking into consideration that K562 cells are Fas-null, we suggested that caspase-8 is activated in a Fas-independent manner downstream of caspase-9. In conclusion, noscapine can induce apoptosis in both apoptosis-proficient and apoptosis-resistant leukemic cells, and it can be a novel candidate in the treatment of hematological malignancies. *Anti-Cancer Drugs* **18:1139–1147 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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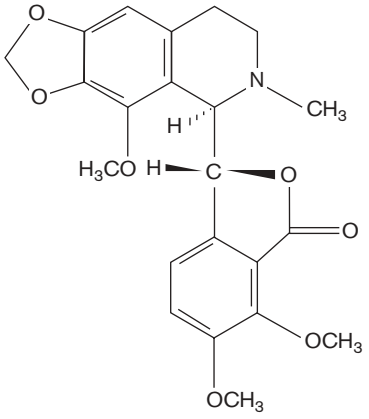
Introduction

Microtubules play critical roles in the maintenance of the shape and motility of cells. The dynamic nature of microtubules is crucial for their organization and function, especially for spindle morphogenesis and chromosome movement during mitosis [1]. Antitumor agents that affect microtubule dynamics are of special medical interest and are commonly used in various chemotherapy regimens [2,3]. Microtubule drugs currently used for cancer treatment include paclitaxel, docetaxel and the vinca alkaloids [4–6]. The administration of these drugs has been hampered by several problems such as (1) poor solubility and toxicity, (2) myelosuppression [7], neuropathy [8] and alopecia [9], and (3) drug resistance developed by overexpression of P-glycoprotein [10], altered expression of tubulin isotypes [11] or the presence of tubulin mutations [12].

Noscapine (Fig. 1) is a phthalideisoquinoline alkaloid from opium, an old antitussive drug [13,14]. It has recently been identified as a microtubule-interfering agent that binds stoichiometrically to tubulin and alters tubulin conformation [15]. Like many other antimicrotubule agents, noscapine suppresses the dynamics of microtubule assembly, blocks cell cycle progression at mitosis and causes apoptotic cell death in many cancer cell types [16,17]. Noscapine does not compete with paclitaxel for tubulin binding, and has antiproliferative effect on both paclitaxel-sensitive and paclitaxel-resistant cells as shown in human ovarian carcinoma cells [18]. Noscapine inhibits the progression of murine lymphoma, melanoma and human breast and bladder tumors implanted in nude mice, with little or no toxicity to the kidney, heart, liver, bone marrow, spleen or small intestine [15,19,20]. It does not inhibit primary immune responses in mice. Noscapine is capable of crossing the blood–brain barrier and inhibiting the growth of implanted rat C6 glioma cells in immunocompromised mice. In this regard, no apparent toxicity to organs with rapidly proliferating tissues or induction of neurological symptoms was observed [21]. Noscapine analogs such as phenol and aniline-6 have a greater antiproliferative effect than noscapine itself [22,23].

In spite of several reports on the efficacy of noscapine to induce apoptosis, data on the mechanism of apoptosis induction have been limited to two reports; necessity of sustained expression of $p34^{cdc2}$ in the FM3A breast

Fig. 1



Chemical structure of noscapine.

cell line for noscapine-induced apoptosis [16] and c-Jun N-terminal kinase (JNK)-mediated apoptosis in response to noscapine in taxol-resistant ovarian cancer cells [18].

Two main activation cascades have been described for the apoptosis induction [24,25]: (1) an external pathway that is mediated by Fas receptor–ligand interaction and caspase-8 as an upstream regulatory caspase, and (2) an internal or mitochondrial pathway that involves the release of cytochrome *c* and the activation of caspase-9. The initiator caspases lead to the activation of executioner caspase-3, -6 and -7, which in turn cleave specific proteins including poly(ADP ribose) polymerase (PARP) and ICAD/DFF45, resulting in changes in the course of apoptosis [26,27]. Several reports suggest that cytotoxic drugs induce apoptosis via CD95/Fas–CD95 ligand interaction [28,29]. In most cases, however, mitochondria are involved in drug-induced apoptosis. Furthermore, some anticancer drugs or radiations can activate CD95/Fas-independent caspase-8, downstream of caspase-3 in cells that are Fas-null [30–34]. A study on the ordering of caspase activation shows that caspase-2, -3, -6, -7, -8 and -9 are processed and activated in response to cytochrome *c* in cell extracts. The same study demonstrated that depletion of caspase-9 from the cell extracts abrogates cytochrome *c*-inducible activation of caspase-2, -3, -6, -7, -8 and -10. This suggests a hierarchical activation of caspase-2, -3, -6, -7, -8 and -10 in a caspase-9-dependent manner [35].

In this study, we investigated the effect of noscapine on apoptosis-proficient myelogenous leukemia HL60 cells and apoptosis-resistant myelogenous leukemia K562 cells, which are resistant to the induction of apoptosis by many stimuli [36].

Materials and methods

Chemicals

Noscapine was obtained from Temad-DP Pharmaco-Chemical Company (Tehran, Iran); RPMI-1640 medium and fetal bovine serum were purchased from Gibco (London, UK); MTT from MP Biomedical LLC (Eschwege, Germany); DAPI (4′-6-diamidino-2-phenylindole), Annexin-V-FLUOS staining kit and proteinase K from Roche (Penzberg, Germany); RNase A from Fermentas (Burlington, Canada); caspase colorimetric protease kit and monoclonal anti-FAS/CD95/APO-1 clone 2R2 from BioSource (Nivelles, Belgium); caspase inhibitor peptides and anti-PARP-1 clone C2-10 from BioVision/Alexis (Lausen, Switzerland); polyclonal anti-caspase-9 from Biomol (Plymouth Meeting, Pennsylvania, USA); ECL Western blotting detection reagent from Amersham Biosciences (Little Chalfont, UK), and protease inhibitor cocktail, phosphatase inhibitor cocktail, phenylmethylsulfonyl fluoride, monoclonal anti-Bcl-2 clone Bcl-2-100, monoclonal anti-Bax clone 2d2, monoclonal anti-β-actin, alkalinephosphatase conjugate goat antimouse IgG, horseradish peroxidase conjugate rabbit antimouse IgG and BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) were purchased from Sigma (Steinheim, Germany).

Methods

Cell cultures and treatment agent

K562 and HL60 cells were maintained in an RPMI-1640 medium, supplemented with 10% fetal bovine serum at 37°C in a 95% air/5% CO_2 atmosphere. The noscapine stock solution was prepared at 0.1 mol/l in dimethylsulfoxide and kept at −20°C.

Cell proliferation assay

The growth-inhibitory effects of noscapine were tested on tumor cells *in vitro* using the MTT assay [37]. About 10^4 K562 and 5×10^4 HL60 cells were seeded in each well of a 96-microwell plate and treated with various concentrations of noscapine. After incubation for 72 h, 20 µl of RPMI-1640 containing 10% fetal bovine serum and 5 mg/ml MTT were added to each well. The plates were then incubated at 37°C for 3.5 h. The precipitated formazan was dissolved in 100 µl of dimethylsulfoxide. Cell viability was evaluated by measuring the optical density of each well at a wavelength of 570 nm, using an ASYS Hitech microplate reader (Asys Hitech, Eugendorf, Austria). The cytotoxicity of noscapine was expressed as IC_{50}, which was calculated using Curve Fit Software (Curve fitting software; 'CurveExpert 1.3', by Daniel Hyams) and presented as mean ± SD of three independent experiments with four replicates for each noscapine concentration.

Assessment of nuclear apoptotic morphology

Nuclear morphology of HL60 and K562 cells was analyzed with DAPI staining. Cells, either noscapine-

treated or untreated, were washed with phosphate-buffered saline (PBS) and stained with 10 μg/ml DAPI. The nuclear morphology of cells was observed by fluorescent microscopy. Cells with condensed or fragmented nuclei were considered to be apoptotic. HL60 cells were analyzed after 6, 18 and 24 h, and K562 cells were analyzed after 18, 24 and 48 h of noscapine administration.

Annexin V staining

Cells were washed in PBS and resuspended in 100 μl of incubation buffer containing Annexin V and propidium iodide according to the manufacturer's instructions. The samples were incubated at room temperature for 10 min and then analyzed by flow cytometry.

DNA fragmentation

Isolation of apoptotic DNA fragments was performed, based on the modified method reported by Walton *et al.* [38]. In brief, the cells were incubated with 20 μmol/l noscapine for 6 and 18 h for HL60 cells, and 24 and 48 h for K562 cells. At the end of incubation, cells were centrifuged, washed twice with ice-cold PBS and a volume equal to 4×10^6 cells of either type lysed on ice for 1 h in 250 μl of 1% Nonidet P-40 and 0.5 mg/ml proteinase K in PBS. The samples were centrifuged, and the supernatant was removed and incubated with 6 μl of 10 mg/ml RNase A at 37°C for 1 h. An equal amount of this sample (20 μl) was electrophoresed through 1% agarose gel containing ethidium bromide in TBE buffer (45 mmol/l Tris-borate, 1 mmol/l EDTA) at 60 V for 2 h.

Caspase activity assay

Activity of caspase-2, -3, -6, -8 and -9 was measured by the colorimetric assay, using a caspase colorimetric protease kit. K562 cells were treated either with 20 μmol/l noscapine alone or pretreated with 5 μmol/l of one of the caspase inhibitor peptides (zDEVD.fmk, zIETD.fmk, zLEHD.fmk and zVAD.fmk; inhibitors of caspase-3, -8 and -9 and of pan-caspases, respectively) 2 h before noscapine treatment. After incubation for 24 and 48 h, the cell lysate was obtained according to the manufacturer's instruction. The cell lysate containing 75 μg of protein was incubated with 4 μl of 4 mmol/l pNA-conjugated substrates (VDVAD-pNA, DEVD-pNA, VEID-pNA, IETD-pNA and LEHD-pNA; substrates for caspase-2, -3, -6, -8 and -9, respectively) at 37°C for 3.5 h. The amount of pNA released was measured at 405 nm using a Microplate Reader LABSYSTEM Multiskan MS (Labsystem, Helsinki, Finland).

Western blotting

HL60 cells were treated with 20 μmol/l noscapine and K562 cells were either treated with 20 μmol/l noscapine alone or pretreated with 5 μmol/l peptide caspase inhibitors (zDEVD.fmk, zVEID.fmk, zIETD.fmk, zLEHD.fmk and zVAD.fmk; inhibitors of caspase-3, -6, -8 and -9 and of pan-caspase, respectively) 2 h before treatment with noscapine. The cells were harvested and rinsed with ice-cold PBS. The cell pellet was resuspended in lysis buffer containing 50 mmol/l Tris–HCl (pH 7.4), 150 mmol/l NaCl, 1% NP-40, 1 mmol/l EDTA, 0.2% SDS, 1% protease inhibitor cocktail, 1% phosphatase inhibitor cocktail and 1 mmol/l phenylmethylsulfonyl fluoride, and left on ice for 30 min. After centrifugation at 12 000 r.p.m. for 10 min at 4°C, the cell lysate was collected and protein concentration was determined according to the Bradford method [39]. Equal amounts of proteins were subjected to 10 and 15% SDS–PAGE (w/v). The proteins were transferred to nitrocellulose membrane, and subjected to immunoblotting using monoclonal anti-Bcl-2 clone Bcl-2-100, monoclonal anti-Bax clone 2d2, anti-PARP-1 clone C2-10, polyclonal anticaspase-9, monoclonal anti-β-actin and monoclonal anti-FAS/CD95/APO-1 clone 2R2 as primary antibodies; alkaline phosphatase and horseradish peroxidase conjugates and goat antimouse and rabbit antimouse IgGs were used as secondary antibodies. Bcl-2 and Bax protein bands related to K562 cells were detected using BCIP/NBT substrate. PARP cleavage and caspase-9 fractionation and also Bcl-2 and Bax protein in HL60 cells were detected by enhanced chemiluminescence using the ECL Western blotting detection reagent.

Statistical analysis

Data obtained in caspase activity assays were evaluated using Student's test for paired samples.

Results

Cytotoxicity of noscapine on myelogenous leukemia cells

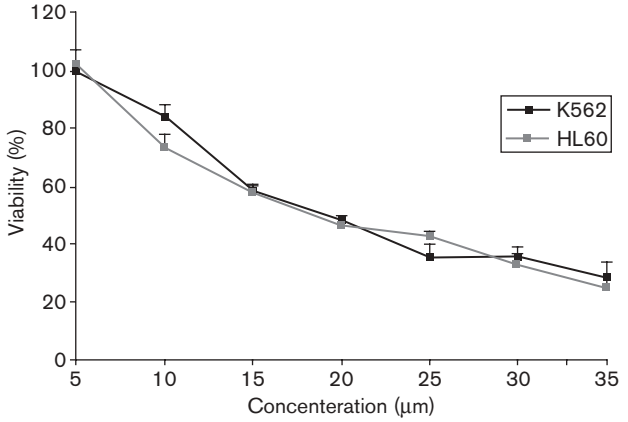
To elucidate the possible apoptotic pathway induced by noscapine, we first examined the survival of apoptosis-proficient HL60 cells and apoptosis-resistant K562 cells treated with various concentrations of noscapine. MTT assays were performed using K562 and HL60 cells in the logarithmic phase of growth, which had been treated with various concentrations of noscapine for 72 h. The survival curve showed that noscapine had a dose-dependent cytotoxic effect on K562 and HL60 cells with IC_{50} equal to 19 ± 1.4 and 19 ± 2.5 μmol/l, respectively (Fig. 2).

Apoptosis induction by noscapine in HL60 and K562 cells

To determine if apoptosis was the major mechanism of noscapine-induced cell death in HL60 and K562 cells, cellular morphological changes, translocation of phosphatidylserine to the cell surface and DNA fragmentation were investigated in these cells.

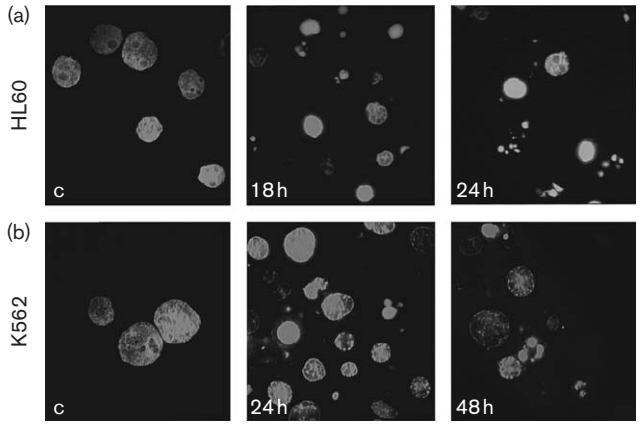
The cells were incubated with 20 μmol/l noscapine for various periods, followed by staining with DAPI and examination by fluorescent microscopy. Noscapine induced marked morphological changes as, for example,

Fig. 2



Cytotoxic effects of noscapine on K562 and HL60 cells. Cells were treated for 72 h in the presence of different concentrations of noscapine. Cytotoxicity was determined by MTT assay. Results are the mean ± SD of three independent experiments.

Fig. 3



Evaluation of morphological changes after noscapine administration. Cells were exposed to 20 μmol/l noscapine and examined for apoptosis by 4′-6-diamidino-2-phenylindole (DAPI) staining. Marked morphological changes such as margination of chromatin, fragmented nuclei and apoptotic bodies appeared in a time-dependent manner in HL60 (a) and K562 cells (b). Magnification, × 100.

marginating of chromatin, fragmented nuclei and formation of apoptotic bodies, which are the typical features of apoptosis. The level of apoptosis gradually increased in both cell lines (Fig. 3). To confirm that noscapine induces apoptosis, we evaluated the percentage of apoptotic cells by Annexin V and propidium iodide staining. The percentage of apoptotic HL60 cells was increased in a time-dependent manner (Fig. 4). DNA fragmentation was also observed in HL60 and K562 cells, which confirmed apoptosis induction in both the cell lines (Fig. 5).

Effect of noscapine on poly(ADP ribose) polymerase cleavage

The cleavage of 116-kDa PARP-1 to 85- and 23-kDa fragments was used as an indicator of apoptosis. In K562 cells, PARP-1 was cleaved partially to the 85-kDa fragment after a 24-h exposure to noscapine. Cleavage was observed to be clearer after 48 h of drug administration (Fig. 6).

Caspase activity in noscapine-induced apoptosis in K562 cells

To examine the mechanism of noscapine-induced apoptosis in K562 cells, we measured the caspase activities using synthetic pNA-conjugated substrates. The activity of caspase-2, -3, -6, -8 and -9 was evaluated after 24 and 48 h of incubation with noscapine (Fig. 7). At these two time points, the activities of initiator caspase-2, -8, and -9 as well as executioner caspase-3 and -6 were elevated significantly ($P < 0.05$).

Impact of caspase inhibition on caspase cascade, poly(ADP ribose) polymerase cleavage and DNA fragmentation

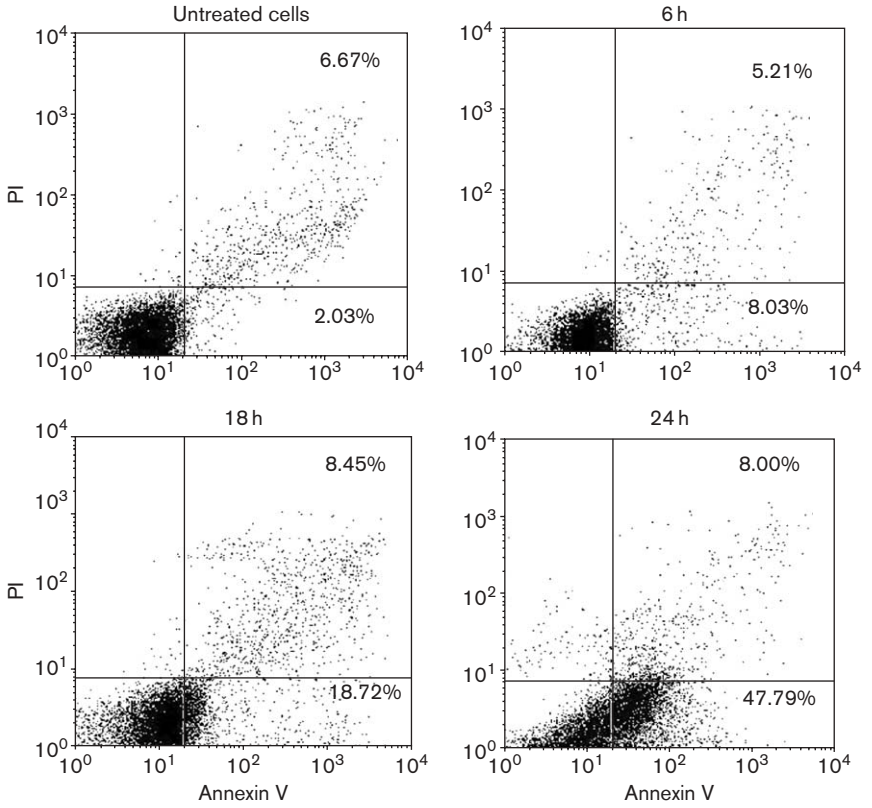
To discern which of the caspases were indispensable for apoptosis in K562 cells treated with noscapine, we tested the protective effect of various caspase inhibitors on PARP cleavage as a hallmark of apoptosis. Inhibitors of caspase-3, -6, -8, and -9 had protective effects on PARP cleavage (Fig. 8).

To clarify which pathway of caspase activation was involved in noscapine-induced apoptosis, we evaluated the activity of caspase-3 and -8 after caspase-9 inhibition, and also the activity of caspase-3 and -9 after caspase-8 inhibition. Inhibition of caspase-9 almost completely blocked the activation of caspase-8 and -3, whereas the inhibition of caspase-8 had a significant effect on caspase-3 activity but no effect on caspase-9 activity (data not shown).

We also checked the processing of caspase-9 in caspase-3 and -8-inhibited samples. ZDEVD.fmk, inhibitor of caspase-3, and ZIETD.fmk, inhibitor of caspase-8, had no effect on caspase-9 processing (Fig. 9).

To investigate probable caspase-independent apoptosis or necrosis, which might manifest after the blocking of caspase-dependent apoptosis, we investigated the DNA fragmentation in K562 cells that were treated either with 20 μmol/l noscapine alone or pretreated with 5 μmol/l pan-caspase inhibitor 2 h before treatment with noscapine (Fig. 10). Noscapine-induced DNA fragmentation changed to smear after caspase inhibition by the pan-caspase inhibitor, which probably is due to necrosis occurring after blocking the caspase-dependent apoptosis.

Fig. 4



Evaluation of apoptosis by annexin V positivity after noscapine administration. HL60 cells were exposed to 20 μmol/l noscapine and examined by flow cytometry after annexin V and propidium iodide (PI) staining at various times. Evidence of apoptotic cells was provided by the binding of annexin V and the retention of PI (lower right quadrant) and double-positive cells underwent secondary necrosis (upper right quadrant). Data are representative of three independent experiments.

Effect of noscapine on Bcl-2 family expression

Bcl-2 and Bax proteins play a pivotal role in controlling cytochrome *c* release and apoptosis initiation via the mitochondrial pathway [40–42]. Our data showed a decrease of Bcl-2 expression level during the first hours of noscapine administration in K562 cells (Fig. 11). The level of Bax protein, however, was not altered significantly upon noscapine treatment at 3–48 h of incubation. Densitometry data showed a 26% increase in Bax/Bcl-2 ratio in the first 3 h of noscapine administration and roughly 60% increase after 48 h compared with untreated K562 cells. Furthermore, the level of Bcl-2 protein after noscapine treatment was investigated in HL60 cells (Fig. 12). In addition to a significant decrease of Bcl-2 level 6 h after administration of noscapine, Bcl-2 phosphorylation was detected after 3 h.

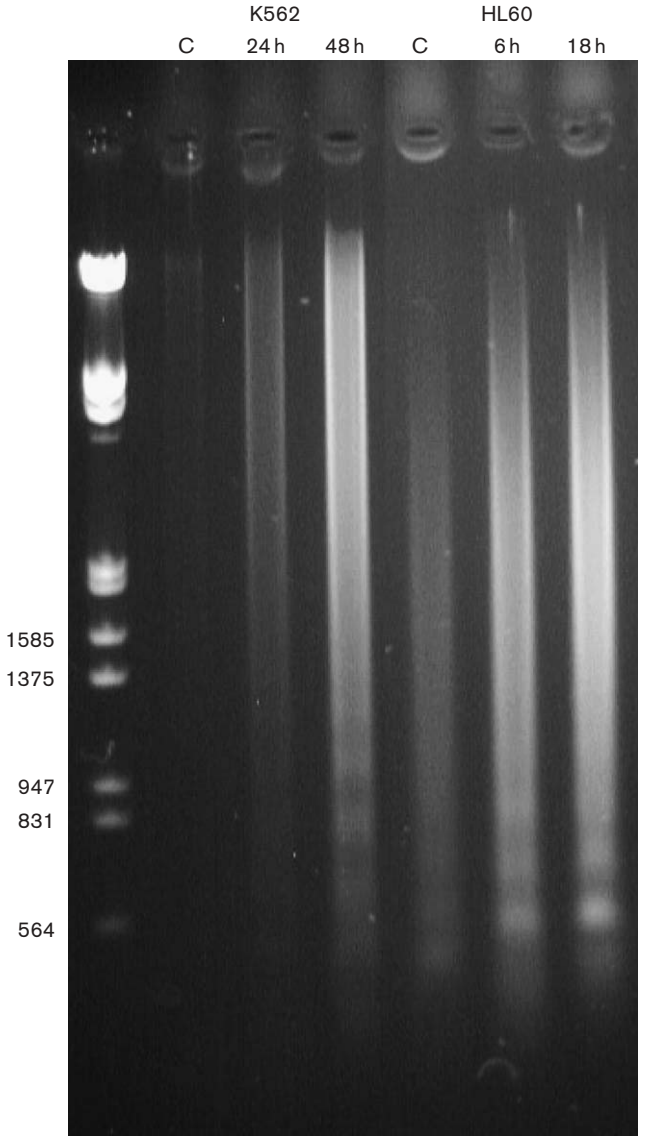
Discussion

Many anticancer compounds used in the clinic are microtubule-related agents. Consequently, considerable efforts have been made over the past decade to develop new related chemicals and to explore their mechanisms. We evaluated noscapine as an anticancer agent in both myelogenous apoptosis-proficient HL60 cells and apoptosis-resistant K562 cells. Noscapine effectively induced apoptosis in both cell lines, with a delay in K562 compared with HL60 cells. A delay in apoptosis induction in K562 cells has been reported upon treatment by etoposide and taxanes [43,44].

In this study, we observed the activation of caspase-2, -3, -6, -8 and -9 accompanied by an increased Bax/Bcl-2 ratio and Bcl-2 phosphorylation in noscapine-induced apoptosis. To investigate the role of caspase cascades in noscapine-induced apoptosis, the effects of different selective caspase inhibitors and pan-caspase inhibitor on the activation of caspase-3, -8 and -9, PARP cleavage, and DNA fragmentation as a hallmark of apoptosis were analyzed. On the basis of these data, we suggested that caspase-3, -6, -8 and -9 all play significant roles in the induction of apoptosis following noscapine treatment.

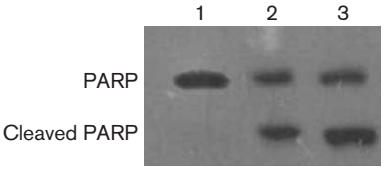
In general, caspase-8 acts as an upstream caspase that relays the death signal generated by Fas receptor–ligand

Fig. 5



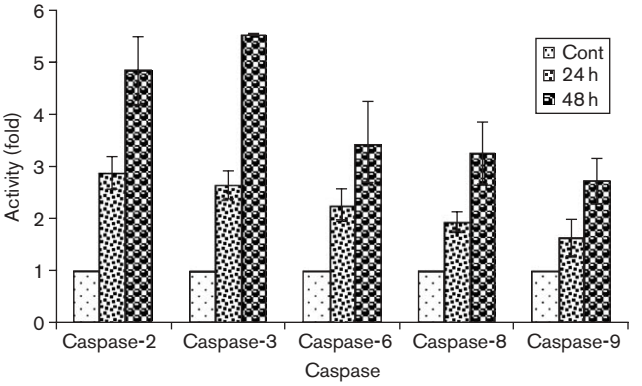
Internucleosomal fragmentation of noscapine-treated K562 and HL60 cells. K562 cells were treated with 20 μmol/l noscapine for 24 and 48 h, and HL60 cells were treated with 20 μmol/l noscapine for 6 and 18 h. After harvesting the cells, isolated DNA was analyzed by agarose gel electrophoresis.

Fig. 6



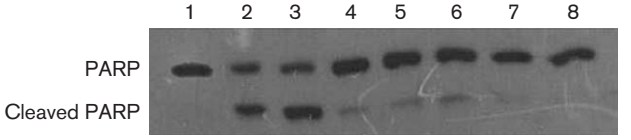
Western blot assay to show cleavage of intact poly(ADP ribose) polymerase (PARP)-1 (116 kDa) to 85-kDa fragments. Lane 1, untreated cells; lane 2, 24 h treatment by 20 μmol/l noscapine; lane 3, 48 h treatment by 20 μmol/l noscapine in K562 cells. Results are representations of three independent experiments.

Fig. 7



Fold activity of caspases after 24 and 48 h of treatment with 20 μmol/l noscapine in K562 cells. Data are presented as mean ± SE and are representative of an average of three independent experiments. Cont, control.

Fig. 8

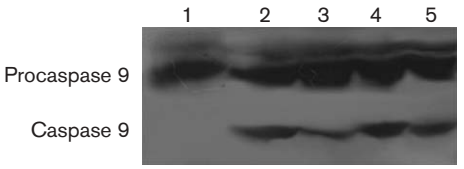


Western blot analysis to show impact of caspase inhibitors on poly(ADP ribose) polymerase (PARP)-1 cleavage. Lane 1, untreated cells; lane 2, PARP-1 cleavage after treatment of K562 cells with 20 μmol/l noscapine for 24 h; lane 3, PARP-1 cleavage after treatment of K562 cells with 20 μmol/l noscapine for 48 h; lane 4, PARP-1 cleavage protected by pan-caspase inhibitor, zVAD.fmk, after treatment of cells with noscapine for 48 h; lanes 5, 6, 7 and 8 show the protective effects of the inhibitors of caspase-3, -9, -8 and -6, respectively, on PARP-1 cleavage after treatment of cells with noscapine for 48 h.

interaction to the downstream caspase-3 and processes procaspase-3 [24]. In the apoptosis induced by anticancer drugs, however, activation of caspase-8 via the Fas receptor–ligand interaction is not an absolute rule [45]. Caspase-8 activity in a Fas-independent manner by several drugs has been reported [30,31,33,46–48]. The results of our experiments confirmed evidence for the complexity of the interplay of different caspases in drug-induced apoptosis. On the basis of the protective effects of inhibitors of caspase-8 and -9 on caspase-3 activity and PARP cleavage, we suggested significant roles both for caspase-8 and -9 in noscapine-induced apoptosis.

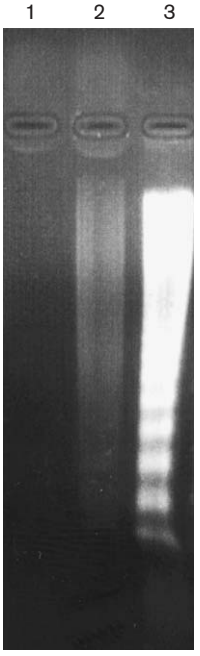
As K562 cells are null for the Fas/CD95/APO-1 receptor [49,50], caspase-8 might become activated via a Fas-independent mechanism in noscapine-induced apoptosis. Besides the lack of Fas/CD95/APO-1 expression in K562 cells, various lines of evidence suggest the activation of

Fig. 9



Western blot analysis to show the processing of 45 kDa of procaspase-9. Lane 1, untreated cells; lane 2, 35 kDa of activated caspase-9 in K562 cells treated with 20 μmol/l noscapine for 24 h; lanes 3, 4 and 5, processing of caspase-9 in cells pretreated for 2 h with 5 μmol/l inhibitors of caspase-9, -8 and -3, respectively, and treated with 20 μmol/l noscapine for 24 h.

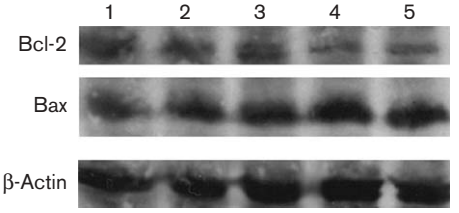
Fig. 10



Agarose gel electrophoresis of DNA extracted from K562 cells to see the effect of caspase inhibition on DNA fragmentation after noscapine treatment. Lane 1, untreated cells; lane 2, cells pretreated with 5 μmol/l pan-caspase inhibitor before addition of 20 μmol/l noscapine; lane 3, cells treated with 20 μmol/l noscapine.

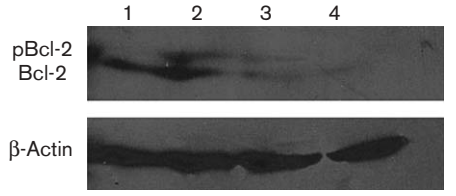
caspase-8 via an internal pathway in our system. Caspase-8 peptide inhibitor zIETD.fmk had no pronounced blocking effect on caspase-9 activation and processing. The weak effect of the caspase-9 inhibitor zLETD.fmk on caspase-9 processing could be due to a lack of specificity of the zLEHD.fmk effect on caspase-9 [32,51]. In contrast, we showed that caspase-8 activity (measured by synthetic substrate) was suppressed by the caspase-9 inhibitor. The use of selective synthetic caspase substrates, however, which are not absolutely specific for caspases, is not the best way to evaluate the caspase cascade.

Fig. 11



Western blot analysis of Bcl-2 and Bax protein expression in K562. Lane 1, untreated cells; lanes 2, 3, 4 and 5, cells treated with 20 μmol/l noscapine for 3, 6, 24 and 48 h, respectively. β-Actin was used as a loading control.

Fig. 12



Western blot analysis of Bcl-2 protein expression and phosphorylation (pBcl-2) in HL60 cells. Lane 1, untreated cells; lanes 2, 3 and 4, cells treated with 20 μmol/l noscapine for 3, 6 and 18 h respectively. Bcl-2 phosphorylation appeared 3 h after noscapine treatment. β-Actin was used as a loading control.

We also showed a decrease of Bcl-2 expression and an increase of Bax/Bcl-2 ratio during the first hours of noscapine-induced apoptosis in K562 cells and a decrease of Bcl-2 protein level accompanied by the phosphorylation of Bcl-2 in the initiation phase of apoptosis induction in HL60 cells. Both the decrease of the Bcl-2 level and the phosphorylation of this protein are pivotal mechanisms triggering the release of cytochrome *c* and the initiation of apoptosis induction via the mitochondrial pathway. Several groups have reported that the administration of antimicrotubule agents induces Bcl-2 phosphorylation via JNK activity in the first stage of apoptosis induction [52,53]. Furthermore, JNK activation upon noscapine treatment has been reported by other investigators [18]. In our experiments, Bcl-2 phosphorylation might have been a consequence of JNK activity. In contrast, a negative regulation of Bcl-2 expression and a positive regulation of Bax transcription by p53, an antitumor protein, have been shown in several studies [54,55]. As both K562 and HL60 cells are p53 deficient, noscapine can induce apoptosis and decrease Bcl-2 expression via the p53-independent pathway.

In conclusion, the fact that about 50% of human cancers harbor p53 mutations [56] along with the observed

apoptotic effects of noscapine in Fas-null apoptosis-resistant leukemic cells has an important implication for developing noscapine either as a chemotherapeutic agent or a dietary supplement to enhance the effects of other anticancer drugs. In-vivo studies on noscapine by other investigators show that noscapine is effective in reducing the growth of lymphoma and increasing the survival of tumor-bearing mice with little or no toxicity to the kidney, heart, liver, bone marrow, spleen or small intestine at tumor-suppressive doses [19]. Other studies showed the inhibitory effects of noscapine on the progression of mice melanoma and human breast and bladder tumors implanted in nude mice. [15,20,57]. In addition, in contrast to the taxanes, oral administration of noscapine was well tolerated by mice. All in-vivo data supports the consideration of noscapine as a candidate for the management of malignancies.

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