Study of Noscapine-Induced Cell Death in Hepatocellular Carcinoma Cell Line

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Summary

Current therapies for cancer treatment are often limited by the emergence of drug resistance and side effects. There is much interest in the identification of new agents for cancer chemotherapy. Noscapine is an isoqiunoline alkaloid found in opium. It is not sedative and has been used as antitussive drug in different countries. Recently, noscapine has been introduced as an anti-mitotic agent. In this study cytotoxic effect of noscapine was evaluated in hepatocellular carcinoma cell line (HepG2). Meanwhile role of apoptosis was explored

Hep-G2 and non-malignant cells (L929) were cultured in RPMI medium and incubated with different concentrations of noscapine (3.75-250 μM) for 24, 48 and 72 h. Paclitaxel used as a positive control. Cell viability was quantitated by MTT assay. Apoptotic cells were determined using Annexine-V/PI staining method.

The results showed noscapine could decrease cell viability in Hep-G2 cells as a concentration and time-dependent manner. The IC50 value against Hep-G2 was determined 75.72 μM after 48 h. Apoptosis was involved in the cytotoxic effect of noscapine.

Thus, apoptosis is involved in noscapine-induced cytotoxicity in Hep-G2 cell line. Noscapine could be considered as a potential chemotherapeutic agent in hepatocellular carcinoma in future.

Keywords: apoptosis, noscapine, cytotoxicity, hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is among the most common cancers worldwide (1). And also it is one of the most common malignancies in Asian countries (2). None of the existing therapies for HCC has shown any promise because of the high frequency of HCC recurrence (3). Therefore, novel strategies to prevent proliferation of malignant cells are urgently needed.

Microtubule-targeting agents such as the vinca alkaloids (vinblastine, vincristine, vindesine, etc.) and taxanes (paclitaxel and docetaxel) are important chemotherapeutic drugs for the treatment of cancer. (4,5) However, increased drug resistance in tumors, (6) poor bioavailability, and poor solubility (7) made scientist to find effective microtubule-directed drugs with improved solubility and therapeutic index.

Noscapine is a naturally occurring phthalideisoquinoline alkaloid obtained from opium with antitussive effect and favorable toxicity profile (8). Recently, it has been known as a weak anticancer agent in certain *in vivo* models.

Noscapine found to effectively inhibit the progression of melanoma (9), murine lymphoma (11), and human breast tumors (12) implanted in nude mice and human breast tumors implanted in nude mice with little or no toxicity to the main organs (13,14). Currently, Noscapine HCl is in phase I/II clinical trials for the treatment of low grade non Hodgkin's lymphoma or chronic lymphocytic leukemia refractory to chemotherapy and hematological malignancies.

Compounds that target microtubules like noscapine can arrest cells at mitosis. Noscapine was found to inhibit cell proliferation in wide variety of cancer cells including many drug-resistant variants (10, 15) while evading normal cells.

In conjunction with previous studies in other types of cancer (9-12, 15-17) it is reasonable to explore the potential use of noscapine for the treatment of hepatocarcinoma cancer. Because of the poor clinical outcome with current treatment options and relatively favorable toxicity profile of noscapine it is important to test Noscapine as an anticancer agent for treatment of HCC.

The induction of apoptosis in tumour cells is considered very useful in the management and therapy as well as in the prevention of cancer. A wide variety of natural substances have been recognized to have the ability to induce apoptosis in various tumor cells (18). It is thus considered important to screen apoptotic inducers in natural compounds (19).

Therefore, in an attempt it is sought to study apoptogenic effects of noscapine in HepG2 cells. HepG2 cells are epithelial-like human HCC cells derived from liver tissue of a 15-year-old Caucasian male HCC is the most common primary malignant neoplasm of the liver worldwide (20,21).

Material and methods

Reagents

The annexin V/PI kit was purchased from Sigma. RPMI and FCS were purchased from Gibco. Noscapine was provided from Sigma.

Cell culture

Cells were obtained from Pasteur Institute (Tehran, Iran). Cells were maintained at 37°C in a humidified atmosphere (90%) containing 5% CO2. Cells were cultured in RPMI with 5% (v/v) fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded overnight, and then incubated with various concentrations of noscapine (3.75- 250 µM) for 24, 48 and 72 h. For MTT assay, cells were seeded at 5000/well onto 96-well culture plates. For assay of apoptosis, cells were seeded at 100,000/well onto a 24-well plate. For each concentration and time course study, there was a control sample which remained untreated and received the equal volume of medium. All different treatment carried out in duplicate.

Cell viability

The cell viability was determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay (22,23). Briefly, cells were seeded (5000/well) onto flat-bottomed 96-well culture plates and allowed to grow 24 h followed by treatment with noscapine (3.75- 250 µM) or Paclitaxel (0.00175-0.175 µM). After removing the medium, cells were then labeled with MTT solution (5 mg/ml in PBS) for 4 h and resulting formazan was solubilized with DMSO (100 µl). The absorption was measured at 570 nm (620 nm as a reference) in an ELISA reader.

Annexin-V-FITC/PI assay of apoptotic cells

Apoptosis was determined by annexin-V-FITC staining and PI labeling, because annexin-V can identify externalization of phosphatidylserine during the progression of apoptosis and, therefore, can detect cells in early stages of apoptosis. HepG2 cells in logarithmic growth phase were seeded in 6-well plate and incubated with Noscapine (100 µM), for 48 h. To quantify apoptosis, prepared cells were washed twice with cold PBS and resuspended in 100 ml binding buffer at a concentration of 1×10^6 cells/ml. Five microliters annexin- V-FITC and 10 ml PI (1 mg/ml) were then added to these cells, which were analyzed with a FACScalibur flow cytometer (Becton Dickinson) and calculated by CellQuest software Early apoptotic cells were positive for annexin-V and negative for PI, while late apoptotic dead cells displayed both high annexin-V and PI labeling.

Statistical analysis

All results were expressed as mean ± SEM. The significance of difference was evaluated with ANOVA and Bonfrroni's test. A probability level of $P < 0.05$ was considered statistically significant.

Results

Effect of Noscapine on cell viability

HepG2 cells and L929 (as non-malignant control cells) were incubated with various concentrations of Noscapine (3.75-250 µM) for 24, 48 and 72 h. Noscapine decreased cell viability in malignant cells but not in non-malignant cells, as a concentration- and time-dependent manner (Figs. 1 and 2). This toxicity was consistent with morphologic changes including reduction in cell volume and rounding (data was not shown). Doses inducing 50% cell growth inhibition (IC50) against HepG2 cells was 75.72 µM.

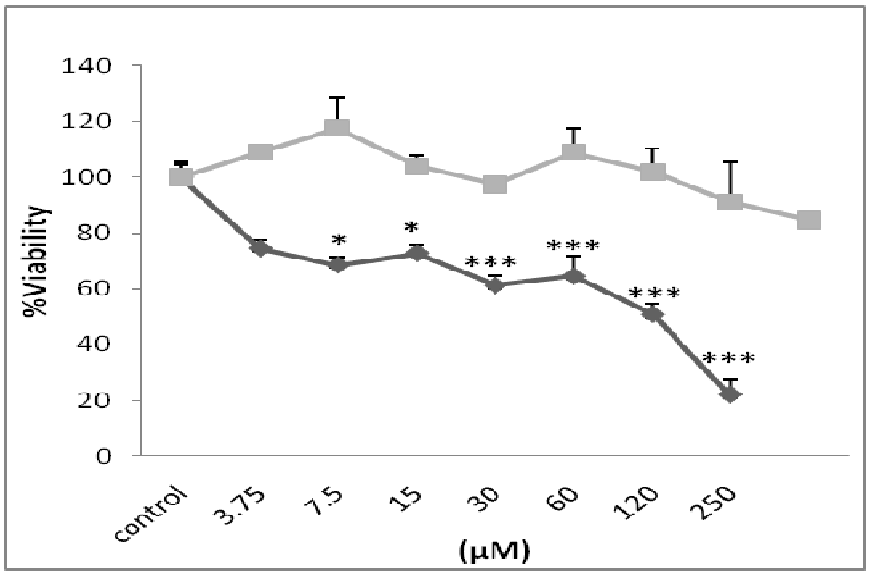

Fig. 1. Comparison of cytotoxic effects of Noscapine on malignant (HepG2) and non-malignant (L929) cells. Cells were treated with different concentrations of Noscapine for 48 h. Results are mean ± SEM (n = 3). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control.

Effect of Paclitaxel on cell viability

We used Paclitaxel as a positive control in this study. HepG2 cells were incubated with various concentrations of Paclitaxel (0.00175-0.175 µM) for 48 h. Paclitaxel decreased cell viability in malignant cells, as a concentrationdependent manner (Fig. 2). This toxicity was consistent with morphologic changes including reduction in cell volume and rounding (data was not shown).

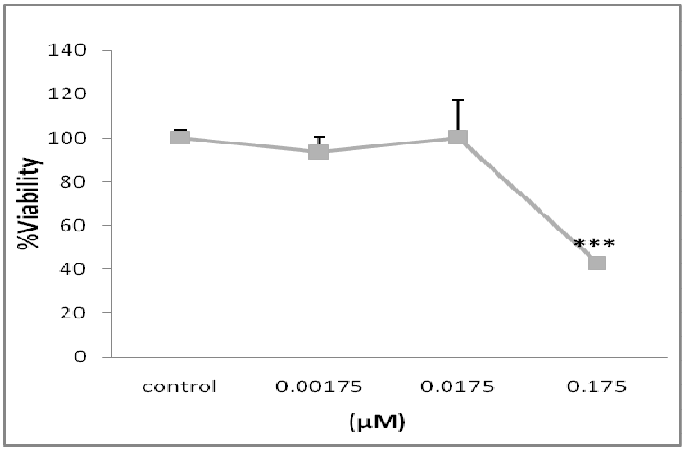

Fig. 2. Effect of Paclitaxel on cell viability of HepG2 cells. Cells were treated with different concentrations of Paclitaxel for 48 h. Viability was quantitated by MTT assay. Results are mean ± SEM (n = 3). The asterisks are indicator of statistical differences obtained separately at different time points compared to their controls shown in figure as ***P < 0.001.

Noscapine Induces Apoptosis in HepG2 Cells

To detect the apoptosis induced by Noscapine, AnnexinV-PI double staining and flow cytometry were used. As shown in Figure 3, Q4 quadrant presented viable apoptosis cells, while Q2 quadrant was non-viable apoptosis cells. The percentage of apoptosis in HepG2 cells induced by of Noscapine (100 µM) for 48 h is 51.69%.

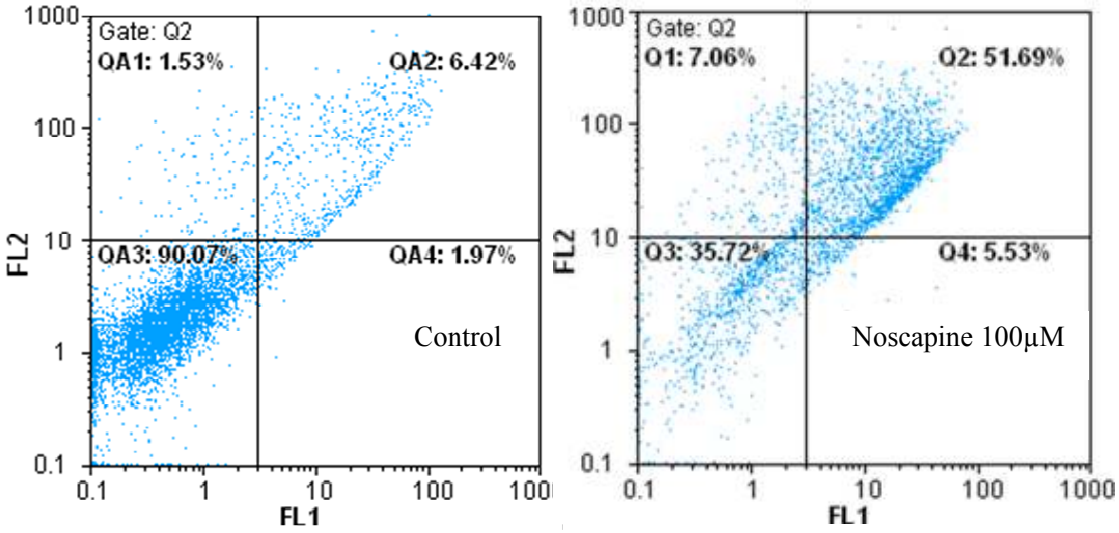

Fig. 3. Noscapine -induced apoptosis in HepG2 cells. Detection of Noscapine -induced apoptosis and necrosis with annexin-V-FITC and PI staining. Exponentially growing cells were treated with the 50 µM of Noscapine for 48 h. Cells with annexin-V and PI staining were measured by flow cytometry. The data represent the mean ± SEM of three independent experiments. Noscapine indicate significant difference from control (**p < 0.01).

Discussion

Cancer is a growing health problem around the world. Natural products have long been used to prevent and treat many diseases, including cancer and thus anti-cancer drugs are developed accordingly (24).

Noscapine is considered as safe antimicrotubule agent that demonstrated antitumor activity both in vitro and in vivo in cancer cells that are resistant to the conventional antimicrotubule drugs. Interestingly, Noscapine don't have side effects that are commonly seen with many chemotherapeutic agents (11, 25-27)

Noscapine has been extensively analyzed for its mechanisms of action (12,16). Similar to other microtubule binding agents, noscapine arrests cells in M-phase and alters the expression levels of cell cycle regulated proteins, such as increased expression of cyclin B1 and survivin and decreased levels of phospho-Cdc2, changes consistent with cells undergoing cell death by mitotic catastrophe (28,29).

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 was reported (16).

In present study, the cytotoxic and apoptogenic effects of noscapine HepG2 cell lines which to our knowledge are the first report on noscapine-induced apoptosis in this cell line were investigated. Our data confirmed that noscapine has cytotoxic activity against HepG2 cell lines more than non-malignant cells tested which is consistent with previous studies indicating that noscapine possesses antitumor and anticarcinogenic activities (9-12,16).

In the present study noscapine -induced apoptosis was involved in induction of cell death. Apoptosis is characterized by distinct morphological features including; chromatin condensation, cell and nuclear shrinkage, membrane blebbing and oligonucleosomal DNA fragmentation (18,30). Apoptosis partially contributed in noscapine-induced toxicity. It might be concluded that non-apoptotic cell death to be also involved in noscapine-induced toxicity in these cells. Although the significance of non-apoptotic cell death in chemotherapy remains, largely unclear, it is believed that the non-apoptotic cell death is important under conditions in which apoptosis is inhibited. (31,32).

In a comparetative study we used paclitaxel as a positive control. The microtubule-stabilizing paclitaxel has ataxane structure that extracted from the Pacific yew tree (Taxus brevifolia) (33). Paclitaxel affinity for microtubules is high and it enhances tubulin polymerization, causing mitosis (M) phase cell cycle arrest (34). Paclitaxel has ability to trigger apoptosis in human Hep G2 cells (35). Paclitaxel-induced apoptosis in HepG2 cells associated with p53 and downregulation of Bcl-xL (36,37).

Taking together, the present study is the first to show toxicity of noscapine in malignant cell lines in which apoptosis or programmed cell death play an important role. It could provide further knowledge to mechanisms involved in this toxicity. noscapine could be also considered as a promising chemotherapeutic agent in HCC cancer treatment.

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