SOHO Supplement 2014

Antiproliferative and Proapoptotic Effects of Topotecan in Combination With Thymoquinone on Acute Myelogenous Leukemia

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Abstract

Topotecan, a camptothecin derivative, is used against different types of cancer. Because of its cytotoxic effect, several approaches have been investigated, combining it with other compounds. Synergism was found in combining topotecan with thymoquinone, exerting a proapoptotic effect through an intrinsic pathway. Pre-exposure of leukemic cells with thymoquinone before topotecan treatment seems to be more effective than adding the compounds simultaneously.

Background: : Topotecan has shown promising antineoplastic activity in solid tumors and acute leukemia. Because of the primary dose-limiting toxicity of topotecan, it is necessary to identify other agents that can work synergistically with topotecan, potentially increasing its efficacy while limiting its toxicity. Many studies showed synergism in combination of topotecan with gemcitabine and bortezomib. Other studies report the increase in growth inhibition of gemcitabine or oxaliplatin when cells were preexposed to naturally occurring drugs such as thymoquinone. The aim of this project was to study the mode of action of topotecan along with thymoquinone, on survival and apoptosis pathways in acute myelogenous leukemia (AML) cell lines, and to investigate the potential synergistic effect of thymoquinone on topotecan. **Materials and Methods:** U937 cells were incubated with different topotecan and thymoquinone concentrations for 24 and 48 hours, separately and in combination. Cell proliferation was determined using WST-1 (Roche) reagent. The effect of the compounds on protein expression of Bax, Bcl2, p53, caspase-9, -8, and -3 was determined using Western blot analysis. Cell cycle analysis was performed in addition to annexin/propidium iodide staining. **Results:** Thymoquinone and topotecan exhibited antiproliferative effects on U937 cells when applied separately. In combination, the reduction in proliferation was extremely significant with a major increase in the expression levels of Bax/Bcl2, p53, and caspase-3 and -9. Preexposure with thymoquinone resulted in an increase in cell growth inhibition compared with topotecan treatment. **Conclusion:** Thymoquinone, when combined with topotecan in noncytotoxic doses, produced synergistic antiproliferative and proapoptotic effects in AML cells. Pre-exposure to thymoquinone seems to be more effective than simultaneous application with topotecan.

Clinical Lymphoma, Myeloma & Leukemia, Vol. 14, No. S3, S46-55
Keywords: AML, Anticancer, Apoptosis, Combined therapy, p53

Introduction

Topotecan (TP) is a novel topoisomerase I (topo I) inhibitor and a water-soluble camptothecin analogue.[1,2] Topoisomerase-1 binds to supercoiled DNA at the 3′-end of the DNA phosphodiester backbone, causing single-stranded breaks during DNA replication and results in the relief of the torsional stresses that are introduced into DNA ahead of the replication complex or moving replication fork. TP induced cell death by stabilizing the covalent complex of topoisomerase and strand-cleaved DNA, thus inducing breaks in the protein-associated DNA single strands.[3,4] It was reported that TP is being evaluated in pediatric cancer patients for treating leukemia, lymphoma, Ewing sarcoma, rhabdomyosarcomas, gliomas, and small-cell and non−small-cell bronchogenic carcinoma, ovarian carcinoma, myeloid leukemia, and small-cell lung cancer.[5-7]

Because of the instability of its lactone ring, TP can undergo reversible hydrolysis to form an open ring with an inactive carboxylate form. This will not only lead to a reduction in potency

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Submitted: Feb 21, 2014; Revised: Mar 26, 2014; Accepted: Apr 3, 2014

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2152-2650/$ - see frontmatter
http://dx.doi.org/10.1016/j.clml.2014.04.014

but also will cause toxic effects such as neutropenia, thrombocytopenia, anemia, and mild nonhematological toxicities like alopecia and fatigue.[6,8-11] It is necessary to identify other chemotherapeutic agents that will work synergistically with TP and that might potentially maintain or increase efficacy while limiting toxicity. Synergism was reported with a combination of TP with bortezomib, irinotecan, and obatoclax mesylate.[2,5,12,13]

Apoptosis is known to be triggered either by extrinsic or intrinsic pathways. The extrinsic pathway can be activated through death receptors like tumor necrosis factor (TNF) receptor, CD95, or TNF-related apoptosis-inducing ligand receptors.[14] The intrinsic pathway is activated by the mitochondrial mediators of caspase-dependent apoptosis which is cytochrome c. When it is released, cytochrome c will activate caspase-3 through formation of the cytochrome c/Apaf-1/caspase-9–containing apoptosome complex.[15]

The effects of *Nigella sativa* seeds have been described in many historical and religious references. *Nigella sativa* is known as a spice that grows in the Mediterranean region and in Western Asian countries including India, Pakistan, and Afghanistan.[16] More than 30% of *N. sativa* seeds are composed of fixed oil and 0.40% to 0.45% volatile oil in which thymoquinone (TQ) represents 18.4% to 24%.[17,18] TQ (2-isopropyl-5-methyle-1,4 benzoquinone) has shown promising beneficial pharmacological effects in treatment of dermatitis, as an antihistamine, antihypertensive, hypoglycemic, antifungal, anti-inflammatory, and antineoplastic compound.[19-25]

Thymoquinone exhibited its antiproliferative effects in many types of cancer: osteocarcinoma and its cisplatin-resistant variant, human breast, human ovarian adenocarcinoma, non–small-cell lung cancer and small-cell lung cancer, and human osteosarcoma cell lines. It has also exhibited cytotoxic effects in colorectal HT29 cells, leukemic cell lines, mouse keratinocytes, papilloma, spindle carcinoma cells, and colon cancer cell line HCT-116 but did not show any toxicity to normal kidney cells or normal human pancreatic ductal epithelial cells.[26-32]

Thymoquinone inhibits tumor angiogenesis and tumor growth by suppressing the activation of Akt (inhibits the phosphorylation of Akt) and ERK; thus it could be used as a potential drug candidate for cancer therapy.[33]

Jafri et al demonstrated that TQ and cisplatin can be considered an active therapeutic combination in non–small-cell lung cancer and small-cell lung cancer cell lines in vitro and in vivo: the combination of TQ and cisplatin have shown a different mechanism of action, TQ being cell cycle-specific and cisplatin non–cell-cycle specific. The combined treatment was more efficient than each compound alone: a 79% reduction in tumor volume was detected. TQ acted synergistically with cisplatin, and caused a decrease in cisplatin resistance by suppressing nuclear factor–κB.[32]

Another in vivo study done by Banerjee et al[26] showed that cells preexposed to TQ, before gemcitabine or oxaliplatin treatments, resulted in 60% to 80% growth reduction, compared with a 15% to 25% reduction when drugs were applied separately. In addition, TQ, in combination with gemcitabine and/or oxaliplatin, exhibited greater antitumor effects in vitro, compared with treatment with the drugs separately. The pretreatment with TQ significantly increased the expression of caspases, resulting in activation of cleaved active component of caspase-3, caspase-9, and the release of cytochrome c, which is an upstream event in activation of the caspase cascade from the mitochondria. In conclusion, the resistance to chemotherapeutic drugs (such as gemcitabine, cisplatin, oxaliplatin, and TP) could be resolved by combining them with TQ.[26] In our study, we aimed to investigate the mechanism of action of TP in the survival and apoptotic pathways in acute myelogenous leukemia as it is used as an adjunct in phase I trials in leukemic patients compared with TQ, and finding a potential synergistic effect of TQ on TP.

Table 1 Different Combinations Applied on the U937 Cell Line for Cell Proliferation Assay

TP (nM)	TQ (μM)
25	5
	10
	15
50	5
	10
	15
75	5
	10
	15

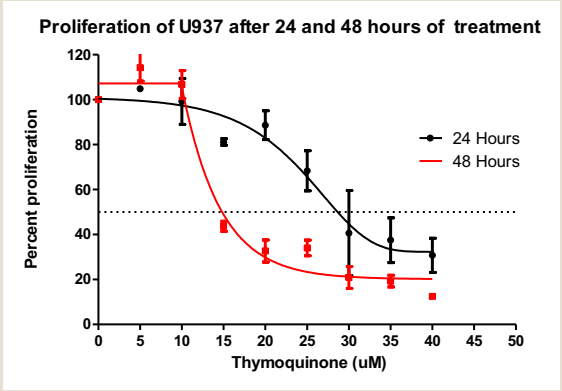
Abbreviations: TP = topotecan; TQ = thymoquinone.

Materials and Methods

Cell Culture

Acute myelogenous leukemia (U937) cells were obtained from American Type Culture Collection and cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) in a humidified atmosphere containing 5% CO_2 at 37°C.

Figure 1 Proliferation of U937 Cells After 24 and 48 Hours of Treatment With Different Concentrations of Thymoquinone (0-40 μM). The Absorbance was Measured at 450 nm After 4 Hours of Incubation With WST-1 (Roche) Reagent. Results Were Normalized to the Untreated Cells. Data are the Mean ± Standard Error of the Mean. U937 Showed a Decrease in Cell Proliferation As Thymoquinone Concentration Increased to Reach Its IC50, Which was 31.27 μM at 24 Hours and 21.39 μM at 48 Hours With $P \leq .0001$



Abbreviation: IC50 = maximal inhibitory concentration.