Thymoquinone in the clinical treatment of cancer: Fact or fiction?

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ABSTRACT

Thymoquinone (TQ) is the bioactive phytochemical constituent of the seeds oil of *Nigella sativa*. *In vitro* and *in vivo* research has thoroughly investigated the anticancer effects of TQ against several cancer cell lines and animal models. As a result, a considerable amount of information has been generated from research thus providing a better understanding of the anti-proliferating activity of this compound. Therefore, it is appropriate that TQ should move from testing on the bench to clinical experiments. The purpose of this review is to highlight the potential of TQ as an anticancer agent and the chances of this compound in the clinical treatment of cancer, with special attention on breast cancer treatment.

Key words: Breast cancer, drug delivery, pharmacokinetics, thymoquinone

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and is spreading further with continuance and increasing incidence in 21st century. Breast cancer is the most prevalent cancer in women. Every year, the world celebrates "pink ribbon day" to spread awareness about this disease. It is one of the main life-threatening diseases that a woman may have to face during her lifetime, with 150 per 100,000 women being diagnosed with this illness each year.[1,2] It comprises 16% of all female cancers and is thought to cause half a million deaths annually.[2] Breast cancer is much less common among men, but it still strikes about 2,000 US males and kills nearly 400 annually.[3]

Several factors that contribute to life-style such as weight gain, obesity, and level of physical activity are associated with breast cancer risk. It was reported that a high intake of meat, dairy products, fat and alcohol may increase the risk and a high intake of fiber, fruits, vegetables, anti-oxidants, and phytoestrogens may reduce the risk of breast cancer.[4] The increasing incidence of breast cancer reported over the last few decades has led to the development of new anticancer drugs, drug combinations and chemotherapy strategies by the scientific exploration of an enormous pool of synthetic, biological, and natural products.[1,5] Experimental investigations demonstrated that many naturally occurring agents and plant extracts have shown anticancer potential in a variety of bioassay systems and animal models.[6-8] *Nigella sativa*, an oriental spice, which is also known as black seeds, has long been used as a natural medicine for treatment of many disease conditions.[9,10] Furthermore, the major bioactive constituent of the volatile oil of black seeds thymoquinone (TQ) [Figure 1] has shown promising pharmacological and therapeutic effects against *in vitro* and *in vivo* disease models.[11] There is an increasing research interest in TQ to evaluate its anticancer activity against breast cancer.[11-13] This coincides with interesting research

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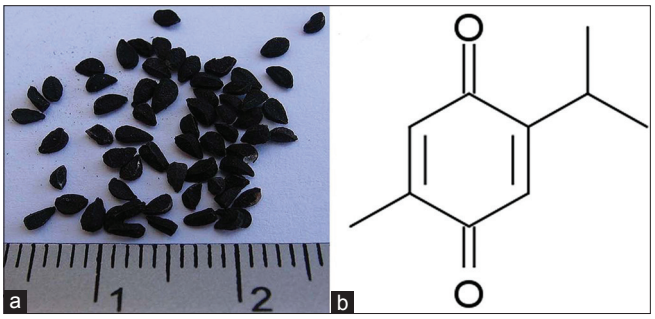

Figure 1: (a) *Nigella sativa* seeds (black seeds). (b) The chemical structure of thymoquinone; 2-isopropyl-5-methyl-1,4-benzoquinone (C10H12O2); Mwt 164.2

findings suggesting new mechanisms of anticancer activity of TQ against breast cancer *in vitro* and *in vivo* models.[14,15] The availability of a review is needed to summarize these findings along with the latest progress in drug toxicity, pharmacokinetics and drug delivery studies of TQ, which could in principle, help in moving into the next level of clinical evaluation of TQ in breast cancer patients.

REVIEW AND DISCUSSION

TQ (2-isopropyl-5-methyl-benzoquinone) is one of the major components of the seeds oil of *N. sativa*. Specific chemical analyses using the high performance liquid chromatography of the seeds oil of *N. sativa* revealed that TQ may attain up to 27.8% of the volatile oil (w/w) composition.[16] TQ has been shown to possess beneficial therapeutic potential on human health as it is evident from many research findings.[11] There is a wide consensus in cancer research that TQ has promising anticancer activity *in vitro* and *in vivo* models.[7,15] It proved to be effective against several types of cancer cell lines in which the classical hallmark of apoptosis such as chromatin condensation, translocation of phosphatidyl serine across the plasma membrane, and DNA fragmentation have been documented in TQ-treated cells.[12] In addition to its apoptotic effect, recent published research revealed interesting inhibitory mechanisms imposed by TQ in breast cancer cell lines MCF-7, MDA-MB-231 and BT-474. Woo *et al*.[17] suggested a new molecular target for TQ anticancer activity against breast cancer cell lines, which is the peroxisome proliferator-activated receptors (PPARs). Three types of PPARs (α, β, γ) have been identified and PPAR-γ is found to play an important role in cell proliferation, differentiation and apoptosis.[18] It was shown to induce G1/S cell cycle arrest by up-regulating $p21^{WAF1/Cip1}$[19] or $p27^{Kip1}$[20] and down-regulating cyclin D1[21] and was reported to control invasion and metastasis of cancer. The authors presented molecular docking studies showing that TQ could make contact with amino-acids within the ligand binding pocket of PPAR-γ that are crucial for its activation. The activation of PPAR-γ was found to play a pivotal role in TQ-induced apoptosis through the activation of caspases and down-regulate PPAR-γ related genes, including Bcl-2, Bcl-xL and survivin at both mRNA and protein expression levels in MCF-7 cells. In addition, TQ was able to reduce the migration and invasion of MDA-MB-231 cells.

The development of multi-drug resistant human tumor cells including doxorubicin-resistant breast cancer cells, provoked further research with TQ to evaluate its effectiveness against this type of cells.[22,23] Arafa *et al*.[23] examined the anticancer effects of TQ in doxorubicin-resistant human breast cancer cells (MCF-7/DOX cells). The authors investigated the potential mechanism by which TQ may regulate cell proliferation and apoptosis in MCF-7/DOX cells. The suggested mechanism is that TQ induces apoptosis in doxorubicin-resistant breast cancer cells through the up-regulation of phosphatase and tensin homolog (PTEN) at the transcription level. The up-regulated PTEN, in turn, inhibits the phosphatidylinositol-3 kinase/Akt pathway and induces p53 and p21 protein expression, thereby causing G2/M cell cycle arrest and apoptosis.

Another potential molecular target for TQ was reported by Connelly *et al*.,[24] which is the nuclear factor kappa B (NF-κB). *In vivo* treatment of TQ on polyoma middle-T oncogene transgenic mouse model resulted in a reduction in tumor volume and weight as compared to the control. Inhibition of NF-κB by TQ increases apoptosis in hyperplastic stages of tumor development and decreases proliferation at least in part by reducing CyclinD1 expression, which inhibits mammary tumor progression.

There is no doubt that cancer research has demonstrated the therapeutic potential of TQ against breast cancer cell lines and animal models with the emphasis on the mechanism of action. In spite of this, there is however a lack of clinical studies testing TQ in human patients with breast cancer. In general, in the process of drug discovery a potential lead compound has to undergo preclinical evaluation prior to clinical trials. This includes understanding of the drug mechanism of action, drug toxicity and determination of its absorption, distribution, metabolism and excretion (ADME) of the drug. Once the ADME is defined, the compound enters the phase of drug development, production/formulation prior to clinical trials.[25] Accordingly, current research has addressed these issues and the use of TQ in clinical settings should therefore be encouraged. With the various mechanisms of action discussed earlier, issues such as: (1) Toxicity of TQ (2) pharmacokinetics of TQ (inclusive of ADME) and (3) TQ delivery will be further discussed herein.

Toxicity of TQ

Many studies were carried out to assess the toxicological properties of TQ *in vitro* and *in vivo*.[26-28] The oral and intraperitoneal median lethal dose (LD50) for TQ in rats as well as in mice were successfully assigned by several research groups.[29,30] The lack of preclinical studies with TQ reporting the maximum tolerated dose (MTD), which is defined as the highest dose that is safe to administer to animal models in the absence of intolerable adverse effects, for TQ toxicity is regarded as a limitation in using TQ in clinical settings to treat cancer. However, preliminary data regarding MTD for TQ in rats suggest lower MTD values in female than in male rats.[31] The possible risk of gender sensitivity to TQ toxicity with female breast cancer patients can be eliminated by adopting combinatorial drug therapy of TQ with doxorubicin, which is commonly used in chemotherapy to treat breast cancer.[32,33] It is worth mentioning that a couple of studies reported that TQ has an antioxidant protective effect against the doxorubicin-induced cardiotoxicity[34] and it does not interfere with the anticancer activity of

doxorubicin[35] in animal models. In addition, using TQ in combination with ionizing radiation such as γ-radiation was found to exert a synergistic cytotoxic effect against breast cancer cells *in vitro*.[36]

Pharmacokinetics of TQ

The lack of bioavailability and pharmacokinetic parameters for TQ delayed the use of TQ in clinical settings. Study the interaction of TQ with blood components reflects the influence on its bioavailability, distribution in the body, metabolism and excretion. Hence, Lupidi *et al*.[37,38] reported the binding interaction of TQ with human serum albumin and Alpha-1 acid glycoprotein (AGP). This was confirmed by using the molecular modeling as well as Fluorescence quenching studies. El-Najjar *et al*.[39,40] studied the effect of TQ binding with bovine serum albumin (BSA) and AGP on its anticancer activity. The results suggested that covalent binding of TQ to BSA lead in losing the TQ anticancer activity against tested cancer cell lines, on the other hand, the TQ anticancer activity was not affected when TQ is bound to AGP. Further investigation is needed for better understanding of TQ pharmacokinetics and for future clinical development.

TQ delivery

The lipophilic nature of TQ introduces a solubility challenge, which could affect its bioavailability and cause limitation in drug formulation. TQ showed to be safe when it is administered orally in several disease animal models. A large number of studies used oral subacute and subchronic TQ in the range of 10-100 mg/kg body weight without any reported toxicity or deaths.[31] However, oral administration of TQ could lead to biotransformation due to induction of phase 2 enzymes in the liver such as DT-diaphorase, a quinine reductase, which catalyzes two electron reduction of TQ into a hydroquinone.[41] Serum protein binding and biotransformation are major factors in affecting TQ anticancer activity *in vivo*. Ravindran *et al*.[42] tested a novel delivery approach for TQ-loaded poly (lactide-co-glycolide) nanoparticles against several cancer cell lines including breast cancer line (MCF-7). The results showed a greater anticancer activity of the encapsulated TQ in nanoparticles than free TQ due to enhanced bioavailability and cellular uptake. Furthermore, Ganea *et al*.[43] tested the anticancer activity of TQ-loaded poly (lactide-co-glycolide) nanoparticles against other breast cancer cell line (MDA-MB-231) and showed to be more effective than free TQ. A recent research done by Odeh *et al*.[44] tested TQ-loaded liposomes particles against MCF-7 breast cancer cell line and proved to be effective in inhibiting the proliferation in this cell line. These results confirm the protective effect of the delivery vehicle against serum protein binding and biotransformation of TQ allowing better anticancer activity.

CONCLUSION AND FUTURE RECOMMENDATION

As per the discussion above, preclinical research results encourage the use of TQ in clinical settings. Considerable amount of information about TQ regarding its molecular anticancer activity, drug toxicity, bioavailability and pharmacokinetics and novel drug delivery approaches are now available for researchers. Al-Amri and Bamosa[45] investigated the effectiveness of oral TQ as an anticancer drug in advanced cancer patients including breast cancer patients. Patients with breast cancer were under treatment for 2 weeks with 400 mg/day. The authors reported neither toxicity nor therapeutic response. This study can be optimized and taken further with either the use of combination therapy of TQ with, for example, doxorubicin or the use of TQ-loaded nanoparticles to treat cancer patients. Moreover, TQ analogs such as caryophyllyl and germacryl conjugates as well as fatty acid conjugates showed a potent anticancer activity against sensitive and resistant MCF-7 breast cancer cell lines.[46,47] Therefore, these analogs can also be tested clinically to evaluate their anticancer activity in breast cancer.

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