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Review on Molecular and Therapeutic Potential of Thymoquinone in Cancer

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Abstract

Thymoquinone (TQ) is the predominant bioactive constituent present in black seed oil (*Nigella sativa*) and has been tested for its efficacy against cancer. Here, we summarize the literature about TQ's molecular mechanism of action and its ability to induce apoptosis and inhibit tumor growth in preclinical models. TQ has anti-inflammatory effects, and it inhibits tumor cell proliferation through modulation of apoptosis signaling, inhibition of angiogenesis, and cell cycle arrest. Chemosensitization by TQ is mostly limited to in vitro studies, and it has potential in therapeutic

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strategy for cancer. The results favor efficacy and enhancement of therapeutic benefit against tumor cells resistant to therapy based on cellular targets that are molecular determinants for cancer cell survival and progression. There have been attempts to synthesize novel analogs of TQ directed toward superior effects in killing tumor cells with more enhanced chemosensitizing potential than parent TQ compound. Based on published reports, we believe that further in-depth studies are warranted including investigation of its bioavailability and Phase I toxicity profiling in human subjects. The results from such studies will be instrumental in advancing this field in support of initiating clinical trials for testing the effects of this ancient agent in cancer therapy.

INTRODUCTION

Thymoquinone (TQ) is the bioactive compound derived from black seed (*Nigella sativa*) oil. It is a spice that grows in the Mediterranean region and in Western Asian countries including India, Pakistan, and Afghanistan. In folklore medicine, the seed is reportedly associated with diverse therapeutic benefits to bronchial asthma, dysentery, headache, gastrointestinal problems, eczema, hypertension, and obesity. We recently reported the therapeutic and chemopreventive potential of black seeds as well as the chemosensitizing efficacy of TQ to gemcitabine and oxaliplatin in pancreatic cancer (PC) (1,2). As further corollary to our previous publications, we succinctly present in this review the therapeutic potential of TQ delineating molecular perspective in the context of cancer. TQ has been reported to exhibit antiproliferative effects on cell lines derived from breast, colon, ovary, larynx, lung, myeloblastic leukemia, and osteosarcoma (3–8) and inhibited hormone refractory prostate cancer by targeting androgen receptor and transcription factor E2F (9). Mechanistically, as proposed by us and others, TQ reportedly induces apoptosis in tumor cells by suppressing NF-κB, Akt activation, and extracellular signal-regulated kinase signaling pathways and also inhibits tumor angiogenesis (2,10–12). As further innovation to our published report on chemosensitization effects of TQ, we present evidence for the sensitizing effect of TQ in gemcitabine resistant PC cells, hitherto unreported and strongly supporting our belief that TQ could be a putative adjunct to conventional chemotherapeutics despite limited studies to date.

ANTIOXIDANT ACTIVITY

According to a provocative view, TQ is a short-chain ubiquinone derivative that potentially acts as a pro-oxidant (13,14). It has been hypothesized that inflammation and pro-oxidant milieu is actually something that is our bodies own creation, an adverse byproduct effect of the essential metabolism and inflammatory system that protects us against diseases. Approximately 20% of all human cancer in adults result from chronic inflammatory state or have inflammatory etiology (15). Interestingly, the effect of TQ in ameliorating oxidative damage and tissue inflammation has been cited in several reports. Its broad spectrum antioxidant potential is associated with its potential to alter “redox state” and its scavenging ability against free radicals, including reactive oxygen species (ROS; superoxide anion radical, hydroxyl radical’s, hydrogen peroxide, peroxynitrate) through modulation of hepatic and extra hepatic antioxidant enzymes such as superoxide dismutase, catalase, and GPx (16,17). Additionally, TQ reduces the cellular oxidative stress by inducing glutathione

(GSH) under different experimental conditions. A substantial body of information exists, implicating a pathological state mediated through induction of lipid peroxidation. Thus far, studies cited in the literature have suggested that TQ protects the kidney against ifosfamide, mercuric chloride, cisplatin, and doxorubicin-induced damage by preventing renal GSH depletion and antilipid peroxidation product accumulation, thereby improving renal functioning (18–20). TQ supplementation in diet of Sprague-Dawley rats inhibited malonaldehyde content in liver, which suggests reduction in the lipid peroxide formation (21). Furthermore, TQ ameliorated hepatotoxicity of carbon tetrachloride as seen by the significant reduction of the elevated levels of serum enzymes and significant increase of the hepatic GSH content (22–24). Khattab and Nagi (25) assessed the protective effects of TQ after chronic inhibition of nitric oxide (NO) synthesis with N (omega)-nitro-l-arginine methyl esters (l-NAME) and found that treatment with TQ increased GSH to normal levels and inhibited the in vitro production of superoxide radicals, thereby offering protection against l-NAME-induced damage possibly via antioxidant properties. Another study by Nagi et al. (24) evaluated TQ as a substrate for mice hepatic DT-diaphorase in the presence of reduced form of nicotinamide adenine dinucleotide (NADH) wherein TQ was found to undergo reduction to dihydrothymoquinone (DHTQ) which turned out to be more potent than TQ and butylated hydroxytoluene (BHT). In a related context, another study reported by Nagi and Almakki (26) found TQ effective in increasing the activities of GSH transferase along with quinone reductase (QR) in mouse liver. This suggests TQ as a promising prophylactic agent against chemical carcinogenesis and toxicity. Another pertinent study reported by Badary and Gamal El-Din (27) found the inhibitory effects of TQ in a fibrosarcoma animal model concomitant with significant reduction in hepatic lipid peroxides and increased GSH and enzyme activities of GST and QR compared to the control group, indicating its potential as a powerful chemopreventive and/or therapeutic agent. The serum/glucose deprivation (SGD)-induced cell death in cultured phaeochromocytoma (PC-12) cells represents a useful in vitro model for the study of brain ischemia and neurodegenerative disorders. The protective effects of *N. sativa* and TQ on cell viability and ROS production in cultured PC-12 cells were investigated under SGD conditions by Mousavi et al. (28). The experimental results suggest that *N. sativa* extract and TQ protects the PC-12 cells against SGD-induced cytotoxicity via antioxidant mechanisms mediated by inhibition of the intracellular ROS production.

ANTI-INFLAMMATORY AND CHEMOPREVENTIVE ACTIVITY OF TQ

Relevant to cancer and inflammation, COX-2 is an important component of the inflammatory response as well as an early response gene whose upregulation leads to the production of multiple prostaglandins (PGs). As a consequence, PGs promote tumor growth by multiple protumorigenic mechanisms such as by stimulating angiogenesis, promoting proliferation, promoting cell invasion, and inhibiting apoptosis through the activation of different oncogenes including v-src, vHa-ras, human epidermal growth factor receptor 2/neu and Wnt (29,30). The first indication of the effect of TQ on in vivo production of PGs and lung inflammation in a mouse model of allergic airway inflammation was reported by El Mezayen et al. (31). Similar to the COX-2 enzyme, another enzyme, 5-lipooxygenase (5-LOX), converts the precursor arachidonic acid molecules to hydroxyleicosaterraenoic acids

or leukotrienes (LT), which in turn enhance proliferation and survival and restrains cells to undergo apoptosis. Therefore, the potential of TQ in suppressing inflammation through inhibition of LT is worth considering as a provocative area to explore in the context of cancer. TQ is reported to be a potent inhibitor of LT formation in human and rat blood cells, showing its effect on 5-LOX and LT-C4-synthase activity (32,33). Another related study reported by El Gazzar (34) showed that TQ, by blocking GATA transcription factor expression and promoter binding, inhibits LPS-induced proinflammatory cytokine (IL-5 and IL-13) production in rat basophilic leukaemia mast cell cells. Amelioration of the inflammation by TQ has been reported by El-Gouhary et al. (35) who found a potent effect of TQ presumably occurring via induction of GSH. We also reported recently that TQ induces inhibition of PGE_2 and COX-2, in a COX-2 overexpressing HPAC cells (PC cells). The anti-inflammatory activities of thymoquinone in pancreatic ductal adenocarcinoma cells have been described by Chehl et al. (10). Available information reveal inhibition of different proinflammatory cytokines and chemokines (monocyte chemoattractant protein-1, TNF-α, IL-1β, and COX-2) by TQ, and the effect was more dramatic than a specific HDAC inhibitor-Trichostatin A (10). Taken together, these studies have collectively supported the notion that TQ could be useful in intervening the inflammatory cascade, which may lead to the inhibition of cancer progression and thus improve patients' morbidity and mortality (2).

Redox-Sensitive Transcription Factor NF-κB

Based on current information, it is estimated that at least 15% of all solid tumors are being driven by NF-κB as a key player. Compelling evidence has also emerged from several research groups that has led to the widely held belief that most cancer preventive agents are NF-κB inhibitors. As reported by us and others, NF-κB is a molecular target of TQ in cancer (2,12). Furthermore, Sethi et al. (12) reported the suppression of TNF-α induced NF-κB activation by TQ in a dose- and time-dependent manner, and TQ also inhibited NF-κB activation induced by various carcinogens and inflammatory stimuli. Of interest, NF-κB activation correlated with sequential inhibition of the activation of IκBα kinase, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and the NF-κB dependent reporter gene expression. TQ specifically suppressed the direct binding of nuclear p65 and recombinant p65 to the DNA, and this binding was reversed by DTT (12). However, TQ did not inhibit p65 binding to DNA when cells were transfected with the p65 plasmid containing cysteine residue 38 mutated to serine, which confirms the specificity of its binding activity (12). We have also reported that TQ is effective in downregulating chemotherapeutic gemcitabine- and oxaliplatin-induced NF-κB activation in PC cells (2). Prior to these extensive reports, Mohamed et al. (36) and Sayed and Morcos (37) had reported the inhibitory effects of TQ on activation of NF-κB in experimental autoimmune encephalomyelitis and human proximal tubular epithelial cells (pTECs) stimulated with advanced glycation end products (AGEs). A significant reduction of AGE-induced NF-κB-activation and IL-6 expression was observed, which points to the potential antioxidative qualities of TQ. In another study, the authors reported that TQ had no effect on the expression of AP-1 protein subunits, c-Jun and c-Fos, but markedly reduced the transcription of GATA-1 and GATA-2 genes (34). Collectively, the preceding results strongly suggest that further in-depth research on the role of TQ in modulation of transcription factors is warranted.

Antiproliferative Activity of TQ

Of particular relevance to pathophysiology of tumor growth is proliferation of tumor cells. Thus far, TQ has been shown to inhibit cell proliferation in cultured cells derived from human breast and ovarian adenocarcinoma (4), myeloblastic leukemia cells, HL-60 (8), squamous carcinoma [SCC VII; (38)], fibrosarcoma [FSSaR;(38)], laryngeal neoplastic cells-Hep-2 (39), and prostate and pancreatic cancer (PC) cell lines (2,9,11,40). Interestingly, human pancreatic ductal epithelial cells (HPDE) cells, noncancerous (BPH-1) prostate epithelial cells, normal kidney cells, and normal human osteoblasts are relatively resistant to inhibitory effect of TQ as reported by us and others (2,6,9). With respect to p53 mutational status, Roepke et al. (6) evaluated the antiproliferative and proapoptotic effect of TQ in two human osteosarcoma cells lines—p53-null MG63 cells and p53-mutant MNNG/HOS cells—and concluded that despite differential involvement of the mitochondrial pathway in inducing apoptosis in these two cell lines, TQ functioned in p53-independent manner in inducing apoptosis in these human osteosarcoma cell lines. This highlights the potential importance of TQ in the clinical setting for the treatment of this cancer because loss of p53 function is frequently observed in osteosarcoma patients. In addition to the study reported by Kaseb et al. (9) that suggested that TQ is a chemopreventive agent for prostate cancer, Richards et al. (41) reported the effectiveness of TQ in retarding the growth of androgen dependent human prostate cancer cell line lymph node carcinoma of the prostate (LNCaP). The beneficial effect of TQ as a neuroprotective agent in inhibiting viability of human neuroblastoma cell line SH-SY5Y in conjunction with L-dopa was explored and reported by Martin et al. (42). We, along with others, reported the anticell viability of TQ in PC cell lines with differences in molecular signatures related to K-ras oncogene status and concluded that TQ inhibits cell proliferation independent of the k-ras mutation status (2,40). According to Womack et al. (39), a single dose of 5 μM of TQ caused a 50% reduction in laryngeal carcinoma Hep-2 cell numbers after 24 h, and a fourfold decline in cell number after 48 h. These results clearly attest the ability of TQ in a sub-therapeutic dose to alter cellular viability. As a spin-off to the antiproliferative effect of TQ, McDermott et al. (43) evaluated the potential of TQ against an important industrial solvent and ambient air pollutant n-hexane induced toxicity and proliferation in Jurkat T-cells. They found that TQ not only inhibited cell proliferation but also significantly reduced n-hexane-induced LDH leakage to the control levels (43). Additionally, Gali-Muhtasib et al. (44) reported suppression of C26 mouse colorectal carcinoma growth in three-dimensional spheroids with significantly increasing signs of apoptosis and 50% decreased C26 cell invasion by TQ.

Cell Cycle Regulation by TQ

Evidence so far indicates the effectiveness of TQ in arresting tumor cells at different stages of their progression. The progression of the cell cycle through the 4 phases of G1, S, G2, and M is regulated by cyclin dependent kinases (CDK) molecules and cyclins, which drives the cell from one phase to the next. Despite limited studies reported for cell cycle regulating protein modification by TQ, it reportedly induces G1 cell cycle arrest in osteosarcoma cancer cells (COS31) as well as in human colon cancer cells (HCT-116), which correlates with reduced expression of CDK inhibitor p16 and downregulation of cyclin D1 (4,5). In

HCT-116 cells, Gali-Muhtasib et al. (5) conducted an extensive study and reported that G1 arrest was associated with upregulation of $p21^{WAF}$, which suggested the principal transcriptional target of TQ is p53 in the context of the G1 checkpoint arrest. It has been hypothesized that the resulting upregulation of $p21^{WAF1}$ blocks cdk2 activity and possibly cdk4 and cdk6 activities leading to G1 arrest. In another study reported by Kaseb et al. (9) in androgen dependent LNCaP prostate cancer cells, TQ caused a dramatic increase in $p21^{WAF1}$, (Cip1), and p27 (Kip1) and blocked the progression of synchronized LNCaP cells from G1 to S phase, with concomitant reduction in AR, E2F-1, as well as the E2F-1-regulated proteins necessary for cell cycle progression. Furthermore, Western immunoblot performed on harvested C4–2B derived tumors in nude mice treated with TQ revealed a dramatic decrease in AR, E2F-1, and cyclin A (9). These results suggest that TQ may prove to be an effective agent in treating hormone-sensitive and hormone-refractory prostate cancers with a reasonable degree of selectivity and possibly other cancers as well. Intriguingly, TQ also causes upregulation of p53 expression. Since virtually all human tumors harbor either deregulated pRB or p53 pathway, or sometimes both, the unique effects of TQ on p53 protein clearly warrant further studies in determining the precise molecular targets of TQ (45). In spindle cell carcinoma, TQ induced growth inhibition by inducing G2/M cell-cycle arrest, which was associated with an increase in p53 expression and downregulation of cyclin B1 protein. However, further studies are highly desirable to investigate the effects of TQ on other proteins that are involved in G2-M transition in order to delineate the molecular mechanism(s) by which TQ may function as an inhibitor of cell cycle progression and thus as an antitumor agent. In PC cells HPAC, TQ pretreatment led to increased cell population at the G0–G1 phase following gemcitabine treatment, whereas oxaliplatin treatment augmented S phase arrest while the proportion of G2-M phase cells decreased (2). Taken together, these studies indicate that TQ pretreatment potentiates the arrest of cells in the progression of the cell cycle.

TQ and Apoptosis

Tumor cells tend to elude apoptosis by deregulating genes that perpetuate programmed cell death (apoptosis). Several studies to date, mostly limited to in vitro cell experiments, document TQ-mediated apoptosis by regulating multiple targets in the apoptotic machinery. Although evidence for reduced cell viability has been observed in response to TQ treatment in breast, colon, bone, leukemia, larynx, prostate, and PC cells, the classical hallmark of apoptosis such as chromatin condensation, translocation of phosphatidyl serine across plasma membrane, and DNA fragmentation have been documented in TQ-treated cells. Furthermore, TQ has been shown by us and others to activate the mitochondrial/intrinsic pathway that involves release of cytochrome c from the mitochondria into the cytosol, which in turn binds to the apoptosis protease activation factor-1 (Apaf-1) and leads to the activation of the initiator caspase-9. Activation of caspase-9 has been described following exposure of human myeloblastic leukemia HL-60 cells (8) and PC cells to TQ (2). Thus, one mechanism of apoptosis induction by TQ involves interference with mitochondrial integrity. There have been no studies reported implicating TQ with the activation of the death receptor/extrinsic pathway of apoptosis in cancer cells. Another molecular entity closely linked to apoptosis is the proapoptotic B-cell non-Hodgkins lymphoma-2 (Bcl-2) family of proteins, which includes Bcl-2-associated x protein (Bax) and Bak, which are activated by

TQ pretreatment (2,45). In several cell lines, prolonged incubation with TQ showed induction of apoptosis by upregulation of proapoptotic Bax protein along with downregulation of antiapoptotic Bcl-2 proteins, resulting in an enhanced Bax/Bcl-2 ratio. In a recent study reported by Gali-Muhtasib et al. (14), checkpoint kinase 1 homolog (CHEK1), a serine/threonine kinase, has been pointed out as one of the targets of TQ, leading to apoptosis in p53+/+ colon cancer cells. On comparing the effect of TQ on p53+/+ as well as p53−/− HCT116 colon cancer cells, p53+/+ cells were found to be more sensitive to TQ in terms of DNA damage and apoptosis induction; it was noted that CHEK1 was ninefold upregulated in p53-null HCT116 cells. The results are in agreement with in vivo experimental findings demonstrating that tumors lacking p53 had higher levels of CHEK1, which was associated with poorer apoptosis, advance tumor stages, and worse prognosis. In related context, Alhosin et al. (46) studied the effect of TQ on p53 deficient lymphoblastic leukemia Jurkat cells and found TQ treatment produced intracellular ROS promoting a DNA damage-related cell cycle arrest and triggered apoptosis through p73-dependent mitochondrial and cell cycle signaling pathway. This was followed by downregulation of UHRF1, which prevented epigenetic code replication and hindered the cancer signature to be inherited in daughter cancer cells (46). This highlights a pivotal property of TQ to stimulate cells lacking functional p53 to undergo apoptosis through a p73 component of signaling cascade when p73 is expressed.

In a recent published study by Torres et al. (47), the expression of Mucin-4 (MUC-4) was investigated in pancreatic cancer cells and it was found that TQ downregulates MUC-4 expression through the proteasomal pathway and induced apoptosis by the activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase (MAPK) pathways. In agreement with previous studies, the decrease in MUC4 expression correlated with an increase in apoptosis, decreased motility, and decreased migration of pancreatic cancer cells. Accordingly, MUC4 transient silencing showed that c-Jun NH2-terminal kinase and p38 MAPK pathways become activated in pancreatic cancer cells, indicating that the activation of these pathways by TQ is directly related to the MUC4 downregulation induced by the drug (47).

TQ Inhibits Angiogenesis and Endothelial Cell Functions

Of relevance to tumor growth, angiogenesis is a prerequisite for supplying oxygen and nutrients to sustain growth beyond a critical size and metastasis. Using the human umbilical vein endothelial cells (HUVECs) and aortic ring assay as a model of angiogenesis, it was demonstrated that TQ has no direct effect on vascular endothelial growth factor (VEGF) receptor 2 activation, which plays a major role in VEGF-dependent angiogenesis but modulates vessel outgrowth and various steps of angiogenesis. TQ inhibits proangiogenic factor (VEGF)-induced ERK activation, and inhibited tube formation on matrigel and induced dose-dependent decrease in the proliferative activity of endothelial cells (10). Overall, these findings indicate that TQ interferes with all essential steps of neovascularization from proangiogenic signaling to endothelial cell migration and tube formation.

SECONDARY TARGETS OF TQ

The effect of TQ on ubiquitin–proteasome pathway has recently emerged from studies reported by Cecarini et al. (48). This pathway represents a nonlysosomal protein degradation system responsible for degrading both damaged/unfolded proteins dangerous for normal cell growth and metabolism and critical regulatory proteins related to apoptosis, cell cycle regulation, gene expression, carcinogenesis, and DNA repair. The effects of TQ on proteasome functionality both in isolated and in cellular complexes in two human glioblastoma cell lines, U87 MG and T98G, differing in their p53 gene status (U87 MG cells present the wild-type form of p53, whereas T98G cells harbor a single p53 mutation) was investigated (48). In purified 20S complexes, the chymotrypsin- and trypsin-like activities were most susceptible to TQ treatment. In the study that reported using U87 MG and T98G malignant glioma cells treated with TQ and 20S and 26S proteasome activity measurement revealed inhibition of the complex in both cell lines but predominantly in U87 MG cells, accompanied by accumulation of ubiquitin conjugates. Accumulation of p53 and Bax, two proteasome substrates with proapoptotic activity, was also observed, demonstrating that TQ induces selective and time-dependent proteasome inhibition, both in isolated enzymes and in glioblastoma cells, and suggests that this mechanism could be implicated in the induction of apoptosis in cancer cells. Recently Al-Naqeep et al. (49) investigated the regulation of low density lipoprotein receptor and 3-hydroxy-3-methyglutaryl coenzyme A reductase gene expression by TQ rich fraction and TQ in HepG2 cells. It was concluded by the authors that TQ regulates genes involved in cholesterol metabolism by two mechanisms, the uptake of low density lipoprotein cholesterol via the upregulation of the LDLR gene and inhibition of cholesterol synthesis via the suppression of the HMGCR gene.

In Vivo Antitumor Activity

Despite limited studies so far, the antitumor activity of TQ seems promising both for chemoprevention as well as in preventing drug-induced toxicity. Additionally, this compound exhibits some selectivity to cancer cells, since normal cells, human pancreatic ductal epithelial cells (HPDE) and mouse keratinocytes are resistant to the apoptotic effects of TQ (2,50). Below, we systemically review studies reported on the site specific antitumor effect of TQ.

GI Cancers: Colon Cancer

Gali-Muhtasib et al. (44), in 2008, evaluated the therapeutic potential of TQ in two different murine colon cancer models, viz.1, 2-dimethyl hydrazine (DMH), and xenografts model. In the DMH model, TQ was injected intraperitoneally and the multiplicity, size, and distribution of aberrant crypt foci (ACF) and tumors were determined at Weeks 10, 20, and 30. TQ significantly reduced the numbers and sizes of ACF by 86%, and tumor multiplicity at Week 20 was reduced from 17.8 in the DMH group to 4.2 in mice injected with TQ. This effect persisted, and tumors did not regrow even when TQ injection was discontinued for 10 wk; and immunostaining for caspase 3 cleavage in remnant tumors confirmed increased apoptosis in response to TQ.

In the xenograft model of HCT116 colon cancer cells, TQ significantly ($P < 0.05$) delayed the growth of the tumors with increased evidence of apoptosis deduced from TUNEL staining of xenografts tumors supporting the potential use of TQ as a therapeutic agent in human colorectal cancer (44). In another study, Al-Johar et al. (21) reported modulation by TQ of aberrant crypt foci in rats induced by azoxymethane.

Forestomach Cancer

Protection to mice against benzo(a)pyrene [B(a)P] induced forestomach carcinogenesis and chromosomal aberrations (CAs) in bone marrow cells by TQ was reported by Badary et al. (51). From their observation, it was inferred that daily intake of the compound before, after, or during exposure to B(a)P significantly reduced the frequencies of CAs and damaged cells compared to the highly clastogenic activity of B(a)P alone. In addition, tumor incidence and multiplicity was seen inhibited in as much as 70 and 67%, respectively.

Fibrosarcoma

The growth inhibitory and antitumor effects of TQ were further studied by Badary and Gamal El Din (27) in fibrosarcoma induced by 20-methylcholanthrene (MC) in male Swiss albino mice. TQ was found effective not only in significantly inhibiting tumor incidence and tumor burden (34% compared to 100% in control tumor-bearing mice), but it also delayed the onset of MC-induced fibrosarcoma tumors—indicative of chemopreventive action against MC-induced fibrosarcomas.

Ehrlich Ascites Carcinoma (EAC)

Badary et al. (18) considered possible augmentation of the antitumor activity of cisplatin by TQ in Ehrlich ascites carcinoma (EAC)-bearing mice and authenticated that TQ (50 mg/l in drinking water), when given 5 days before and 5 days after single injection of cisplatin, abrogated cisplatin nephrotoxicity and potentiated the antitumor activity of cisplatin. Another study reported by Badary (19) in mice bearing EAC xenograft, documented that TQ (10 mg/kg/day) in drinking water significantly enhanced the antitumor effect of Ifosfamide. Mice treated with Ifosfamide in combination with TQ showed less body weight loss and mortality rate compared to Ifosfamide monotherapy. These observations demonstrate that TQ may improve the therapeutic efficacy of Ifosfamide and cisplatin and in addition, reverses Ifosfamide- and cisplatin-induced nephrotoxicity by preventing renal GST depletion and lipid peroxide generation and improving their antitumor efficacy.

Prostate Cancer

Kaseb et al. (9) observed in a xenograft prostate tumor model that TQ inhibited growth of C4–2B derived tumors in nude mice. This was associated with a dramatic decrease in androgen receptor, transcription factor E2F-1, and cyclin A as determined by Western blot analysis. Their findings clearly suggest that TQ may prove to be an effective agent in treating hormone sensitive, as well as hormone refractory, prostate cancers with reasonable degree of selectivity. TQ was also shown in another study of human prostate cancer (PC3 cells) xenograft to inhibit the tumor growth and block angiogenesis with almost no toxic side effects (10).

TQ AND CHEMOSENSITIZATION OF CANCER

Preclinical studies reveal the potential of TQ in improving the therapeutic effect of anticancer drugs and also protection of nontumor tissues against chemotherapy-induced damages. For example, TQ ameliorated nephrotoxicity and cardiotoxicity by cisplatin, ifosfamide, and doxorubicin (18,52,53). However, no Phase I pilot study has so far been reported evaluating its promising anticancer effect in human subjects. Of interest, Gali-Muhtasib et al. (50) noted that TQ induces a sharp increase in p16 protein levels within 2 hr of treatment; this observation is of interest, since as mentioned by Gali-Muhtasib et al. (54) and Hochhauser (55), modulation of p16 protein expression increases tumor sensitivity to chemotherapeutic drugs.

A study reported by Barron et al. (56) evaluated proliferation of osteoblasts cells (MG 63) following a combined dose of TQ and selenium (Se). Their results revealed reduction in cell proliferation, increased cellular damage, decreased alkaline phosphatase levels, and decreased GST levels, indicating that the combined use of TQ and selenium (Se) may be an effective treatment option against human osteosarcoma cells (56). In a study reported by Worthen et al. (57), TQ and related compounds were assayed in vitro for their cytotoxicity in several parental and multidrug resistant (MDR) human tumor cell lines; and it was inferred that TQ, which exhibits cytotoxicity for several types of human tumor cells, may not be an MDR substrate. We recently reported the chemosensitizing effect of TQ to conventional chemotherapeutic agents both in vitro and as well as in vivo in an orthotopic model of PC. In vitro studies revealed that preexposure of cells with TQ (25 μM) for 48 h followed by exposure to gemcitabine or oxaliplatin resulted in 60–80% growth inhibition compared to 15–25% when gemcitabine or oxaliplatin was used alone. TQ synergized killing of PC cells by the downregulation of NF-κB, Bcl-2 family, and NF-κB dependent antiapoptotic protein members—XIAPs and survivin. It has been previously shown by our laboratory that NF-κB gets activated on exposure of PC cells to conventional chemotherapeutic agents (58,59); interestingly, TQ was able to downregulate NF-κB in vitro, resulting in chemosensitization. In addition, we also reported, for the first time, that TQ in combination with gemcitabine and/or oxaliplatin appears comparatively more superior as an antitumor agent compared to either agents alone, and that NF-κB was found inactivated in tumors that were pretreated with TQ followed by gemcitabine and/or oxaliplatin. These results provide strong in vivo molecular evidence in support of our hypothesis that TQ abrogates gemcitabine or oxaliplatin induced activation of NF-κB, resulting in the chemosensitization of pancreatic tumors to conventional therapeutics.

TQ ANALOGS

Attempts have been made to synthesize novel analogs of TQ with superior efficacy for use against tumors. Effenberger et al. (60) recently reported and evaluated conjugates of TQ with various monoterpenes, sesquiterpenes, and the cytotoxic triterpene betulinic acid for improved anticancer activity. They (60) attached esters of various terpene alcohols to C(6) of TQ via spacers of variable length. The resulting derivatives were tested for growth inhibition of cells of the human cancer cell lines HL-60 leukemia, 518A2 melanoma, MDR KB-V1/Vbl cervix carcinoma, and MCF-7/Topo breast adenocarcinoma and nonmalignant

foreskin fibroblasts. Some of these analogs were far more efficacious in certain cancer cell lines than the parent drug while being considerably less toxic to nonmalignant human fibroblasts. Among the synthesized derivatives, two conjugates revealed the best results of all test compounds against MDR MCF-7 breast carcinoma cells. Their effects on the mitochondrial membrane potential and the cellular levels of ROS were also evaluated and reported as the basis for their cellular mechanism of action (60). Our laboratory also recently reported and evaluated the biological effect of TQ analogs against gemcitabine resistant pancreatic cancer cell line (MiaPaCa-2). Out of 27 analogs synthesized, 3 compounds exhibited superior activity than TQ at equimolar concentration (10 μM). These compounds were further evaluated toward improvement of sensitivity to oxaliplatin and gemcitabine in pancreatic cancer cells and again were found superior to parent TQ in causing reduced cell viability and inducing apoptosis (61). Other related studies reported include a study by Ravindran et al. (62) that reported TQ encapsulated in biodegradable nanoparticulate formulation (based on poly (lactide-co-glycolide) (PLGA) and the stabilizer polyethylene glycol (PEG)-5000) enhanced antiproliferative, antiinflammatory, and chemosensitization potential. Further investigations using a panel of markers directed toward understanding its efficacy toward cell proliferation, metastasis, angiogenesis, and chemosensitization revealed TQ nanoparticles (NP) being more active than TQ. TQ-NP were more potent than TQ in suppressing proliferation of colon cancer, breast cancer, prostate cancer, and multiple myeloma cells and was found to be were more potent than TQ in sensitizing leukemic cells to TNF- and paclitaxel-induced apoptosis (62).

CONCLUSIONS

Based on the foregoing account, the therapeutic potential of TQ should not be undermined despite lack of any study accounting their bioavailability, which needs to be pursued. Once a consensus on its bioavailability emerges, planned Phase I clinical trials to validate its usefulness in chemosensitization and status as chemopreventive agent must be prioritized for different site-specific cancers. Meanwhile, efforts should continue focusing on laboratory research to gain further in-depth understanding on its molecular mechanism of action as well as to devise strategy to devise potent analogs with minimum to low side effects, with the ultimate goal of translating the benefits of this nature endowed compound for therapeutic uses for diseases afflicting humans.

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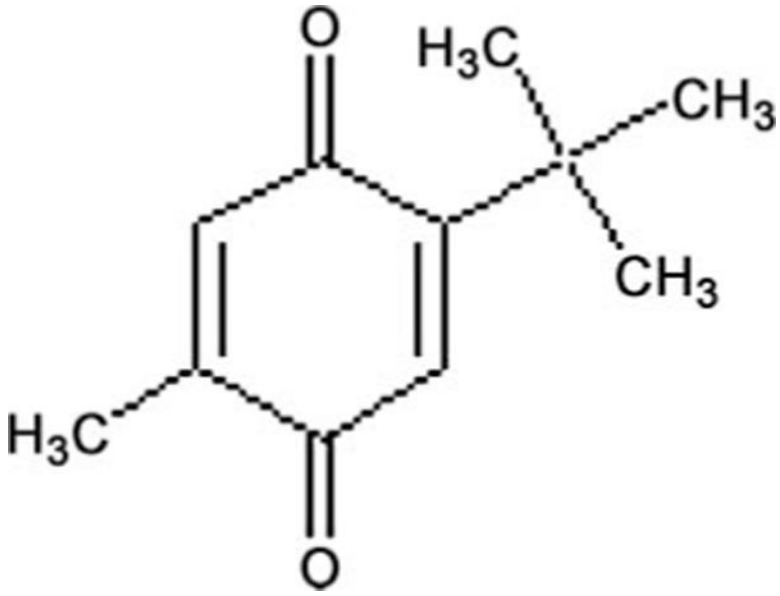

FIG. 1.
Structure of thymoquinone (2-Isopropyl-5-methyl-1,4-benzoquinone).

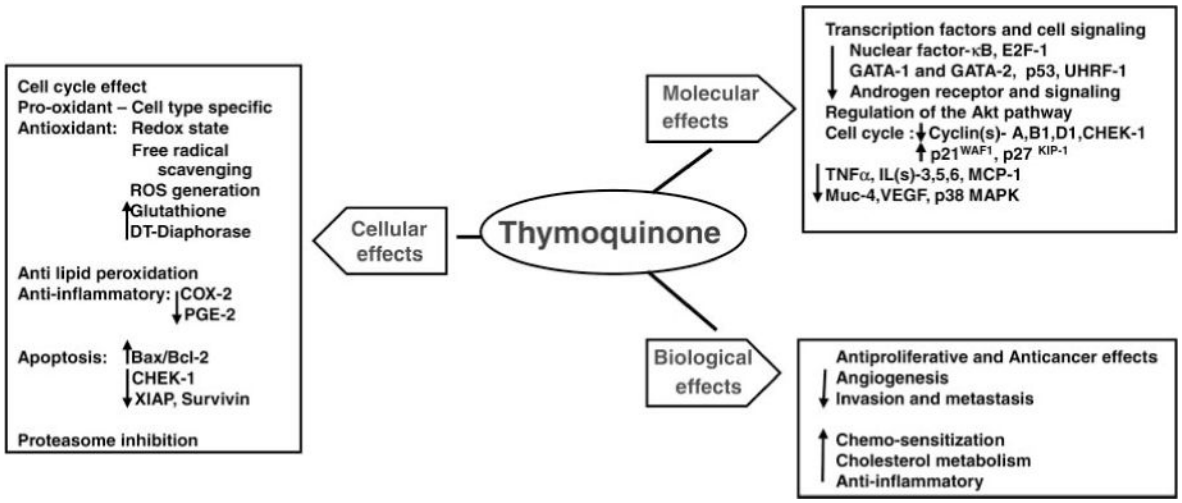

FIG. 2.

Multitargeted effects of thymoquinone. ROS, reactive oxygen species; DT, dehydragenase quinine; COX-2, cyclooxygenase-2, PGE-2; prostaglandin E-2; Bcl-2, B-cell non-Hodgkin lymphoma-2; Bax, Bcl-2-associated x protein; CHEK-1, checkpoint kinase 1 homolog; XIAP, x-linked inhibitor of apoptosis protein; E2F-1, E2F transcription factor 1; GATA, GATA transcription factor; UHRF, ubiquitin PHD RING finger; TNF α, tumor necrosis factor alpha; IL, interleukin; MCP-1, monocyte chemotactic protein-1; Muc-4, Mucin-4; VEGF, vascular endothelial growth factor; MAPK, mitogen-activated protein kinase.