Review

Tamoxifen and oxidative stress: an overlooked connection

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Abstract
Tamoxifen is the gold standard drug for the treatment of breast cancer in pre and post-menopausal women. Its journey from a failing contraceptive to a blockbuster is an example of pharmaceutical innovation challenges. Tamoxifen has a wide range of pharmacological activities; a drug that was initially thought to work via a simple Estrogen receptor (ER) mechanism was proven to mediate its activity through several non-ER mechanisms. Here in we review the previous literature describing ER and non-ER targets of tamoxifen, we highlighted the overlooked connection between tamoxifen, tamoxifen apoptotic effects and oxidative stress.

Keywords Apoptosis · ER · Oxidative stress · Resistance · Tamoxifen

1 Introduction

Tamoxifen (1) is a blockbuster drug and a bestseller for hormonal dependent breast cancer. It is the most commonly prescribed drug for estrogen receptor (ER) positive breast cancer patients. For the past 40 years, it succeeded in saving lives. Tamoxifen successful use for treatment and prevention of breast cancer has allowed it to stand the competition with emerging treatments. Tamoxifen cost effectiveness, made it a golden standard treatment for decades [1]. There is a huge interest not only in tamoxifen but also in its hydroxylated metabolites 4-hydroxy tamoxifen (4OH-TAM) (2) and endoxifen (3), the study of those active metabolites has helped in understanding the mechanism of action of tamoxifen in breast cancer [2].
Tamoxifen is pharmacologically classified as Selective Estrogen receptor modulator (SERM). SERMs are a group of compounds bearing diverse chemical scaffolds; SERMs interact with ERs either agonistically or antagonistically based on the target tissue and physiological context [3].

In 1987, Jordan et al. discovered that tamoxifen and raloxifene perceived then as “antiestrogen” showed estrogenic effects on bones in ovariectomized rats [4]. The discovery of ERβ as another subtype of ER was another reason to revolutionize the idea on nuclear receptor functioning [5].

The oversimplified interpretation of SERM action described by agonist/antagonist was no longer acceptable for nuclear hormone physiology. Therefore, broad insights were needed to understand the molecular basis of ER/SERM actions. An old school interpretation of ER/SERM action is the ligand dependent mechanism, when ligand binds to ER, the heat shock proteins dissociates and the two-receptor subunits dimerize. The receptor dimer then associates with ERE (Estrogen Receptor Element), this in turn recruits and modulates the so-called co-regulatory proteins namely co-activators and co-repressors. Co-activators bridges the Estrogen/ER or SERM/ER complex to the basal transcription machinery leading to histone acetylation and gene transcription whereas co-repressor recruitment leads to the exact opposite action (Fig. 1) [6, 7].

One of the major differences between Estrogen/ER complex and SERM/ER complex is the way they recruit co-activators. E2 interact with AF2 domain of ER and this amplifies the gene expression whereas SERM prevents this interaction and its consecutives transcriptional effects [8].

In some cases, the promoters of estrogen regulated genes may not possess an ERE sequence, so another indirect genomic ligand dependent mechanism takes over. The ER ligand complex binds to transcription factors such as AP-1 and Sp1 leading to gene activation. This mechanism provides some interpretation to the differential activities of Estrogen/ER complex and SERM/ER complex. It is noticed that while a SERM/ER complex can cause activation to AP-1 sites on ERα, they can cause decreased activation through to AP-1 sites on ERβ (Fig. 2).

The ability of ER and SERM to elicit very rapid responses on a wide range of cells and organs is attributed to non-genomic mediated actions. It is postulated that a cell surface ER is probably linked to a G-protein. The non-genomic effects are seen through a cascade of events including secondary messengers [9, 10] (Fig. 3).

The chemical structure of SERM provide some basic interpretation for their dual pharmacological activity. Medicinal chemists invested a lot in researching the differences in chemical structure between full agonists, full antagonists and SERM. These investigations provide an insight of optimum chemical structures that can be used as ideal...
SERMs. A basic pharmacophore for all SERMs is reported, at least two aromatic rings are arranged in a stilbene like arrangement \[11\] and a minimum of one phenolic OH is essential for optimum binding \[12\]. An overlay between co-crystallized 4-hydroxytamoxifen and diethylstilbestrol (DES) showed that the co-crystallized phenol containing ligand shows an important interaction between the OH group of the ligand, a water molecule and Glu 353/Arg 394 residues \[13\] (Fig. 4).

All SERM bear an additional structural feature, a phenyl ring with a bulky substituent at position 4 between the two core phenyls. This protruding substituent moves helix 12 from its agonistic conformation and displaces it from the receptor opening. When the receptor binds to an agonist, helix 12 hydrophobic groove becomes exposed to the nuclear box recognition sequence containing the key LXXLL motif. This sequence is common to the many coactivator proteins that are essential for successful ER transcriptional activity. This agonistic mechanism does not occur when a SERM is bound to the ligand binding domain (LBD), as helix 12 dislocates and occupies the space for the coregulatory protein recognition sequence LXXLL binding \[14\] (Fig. 5).

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**Fig. 3** Non-genomic ligand dependent ER mechanism

**Fig. 4** Overlay of 4-OHTAM in green with DES (red)

**Fig. 5** Helix 12 controlling transcription activity of ER
2 Tamoxifen history and development

Tamoxifen was first given the name: compound ICI 46,474 in 1962 by ICI Company. It was first synthesized as a part of a project that aims to developing contraceptive pills. The failure of the compound to elicit anti-estrogenic effect on the ovary and its ability to stimulate ovulation was a great disappointment to ICI pharmaceuticals. Aiming to save the company's investments, it was suggested to repurpose tamoxifen for breast cancer patients. It received the first approval as a drug for palliative care in late stage breast cancer in 1982. Tamoxifen that started as an orphan drug turned to a bestselling medicine in a journey that started in the eighties. The clinical trials showed its potential use as adjuvant to surgery and chemotherapy in the breast cancer early stages. This was followed by an additional clinical use as a preventive agent in women who are at high risk of developing breast cancer. Tamoxifen was the first chemopreventive agent for any cancer, this extended the market for similar drugs and increased the research focus on chemopreventive agents [15]. By the end of 1970, a relatively small number of breast cancer patients were admitted to clinical trials involving ten weeks treatment with tamoxifen, the tumor regressed in 67% of them in a detectable and clear manner. These findings were similar to results of patients maintained on DES treatment, yet the lack of toxicity and absence of any severe side effects compared to DES boosted the future of tamoxifen [16]. Once tamoxifen was proven effective in both early and late stage breast cancer, many rivals emerged. Tamoxifen yet had two competitive advantages namely lacking androgenic activity and low incidence of side effects.

1975–1980 were the glorious years of tamoxifen discovery project; Clinical trials proved that tamoxifen shows a strong response in old patients with recurrent breast cancer and/or radiotherapy. This action dictated further studies to investigate the ability of tamoxifen or its metabolite to work via non-ER mediated mechanisms. Therefore, tamoxifen was screened in progesterone, androgen and prostaglandin synthetase (PGS) inhibitor screens to find out whether any of these are possible binding targets for tamoxifen [1]. Results showed tamoxifen is a potent inhibitor of PGS that can relieve bone aches in advanced breast cancer patients. This further supported its use in adjuvant chemotherapy and raided the scientific interest in finding new analogues with anti-estrogenic activity and no estrogenic effects and most important with potent PGS inhibitory actions [15]. Clinical data showed that Nolvadex could be used effectively in both pre- and postmenopausal women with ER positive breast cancer tissues. 5–10% of the ER-negative breast cancers have also shown sensitivity to tamoxifen treatment via ER independent signaling pathways [17, 18]. Research findings also highlighted the successful use of tamoxifen in prophylaxis and in remarkably lowering the incidence of recurrence in high-risk patients [19, 20]. The story of tamoxifen from a failing contraceptive to a blockbuster is considered as an inspiring story of pharmaceutical innovation (Fig. 6).

![Timeline of tamoxifen development](image_url)
3 Tamoxifen non-ER targets

At early times, tamoxifen was believed to work via basic ER competitive antagonistic mechanisms. Nowadays, there are immense knowledge to confirm tamoxifen actions are complex and are not limited to its ER modulator properties. Tamoxifen antitumor effect is mainly attributed to the selective blockage of the ERα expressed in some cancer types yet its indications now go beyond breast cancer to many ER-negative tumors. Many researchers were encouraged to investigate new targets for tamoxifen other than ER. Some of the targets investigated included:

3.1 Protein kinase C (PKC)

One of the initial targets investigated was Protein kinase C (PKC). It plays an essential role in intracellular signaling and cell growth regulation. Tamoxifen inhibited PKC in a manner similar to Ro31-8220, a PKC specific inhibitor. PKC inhibition induced p21(waf1/cip1), Rb dephosphorylation and G1/S phase cell arrest [21]. Tamoxifen cytotoxic effect was demonstrated on prostate cancer, hepatocellular carcinoma [22] and astrocytoma [23]. Tamoxifen can bind directly to PKC epsilon (a PKC isotypes that is characterized as a calcium-independent kinase) in MCF-7 cell culture hindering both differentiation and growth [24].

3.2 Metalloproteinase

Tamoxifen successfully up-regulated expression of metalloproteinases-1, TIMP-1, and down-regulated expression of matrix metalloproteinase 9, MMP-9. Inhibition of proteases inhibited cell invasion in the in-vitro assays, tamoxifen inhibited metastasis in both ER-positive like lung adenocarcinoma SPC-A-1 and breast cancer lines MCF-7 [25]. Tamoxifen was also active on ER-negative cell lines like human thyroid cancer cells and B16-BL6 murine melanoma cell lines [26]. The anti-metastatic effect was proven in-vivo in murine model where tamoxifen led to a major inhibition of melanoma metastasis in particular to the lungs [27]. Tamoxifen use led to reduction in tumor size and inhibition of metastasis in Fischer rats [28].

3.3 Vascular endothelial growth factor (VEGF) and angiopoietin-1

Tamoxifen possesses potent anti-angiogenic properties that facilitates its tumor growth and metastasis inhibitory activity in both ER+ and ER- cell lines [29, 30]. Tamoxifen and (4-OHTAM) inhibit platelet activation that consecutively lowers the vascular endothelial growth factor (VEGF) and increases the angiopoietin-1. These two proteins are key players in angiogenesis and metastasis [31] (Fig. 7).

In addition to the aforementioned proteins, reports highlighted the involvement of cyclin-dependent endothelial cell growth [29], transforming growth factor beta (TGF-β) [32] and basic fibroblast growth factor (bFGF) in the antiangiogenic action of tamoxifen and its metabolites [33].

![Fig. 7 Tamoxifen and 4-OHTAM anti-angiogenic mechanism](image-url)
3.4 ABC transporter family

Another tamoxifen target are proteins responsible for chemotherapeutic efflux of antitumor agents, this action is responsible for multidrug resistance mechanism in cancer patients (MDR). The proteins involved are transport proteins of the ABC transporter family [34, 35]. Tamoxifen enhanced the activity of some anti-tumor agents via interaction with P-glycoprotein (Pgp). Tamoxifen competes over P-glycoprotein (Pgp) with cytostatic agents, and therefore leads to a recovery of chemotherapeutic effect in particular with cells having an MDR phenotype [36].

To further validate Pgp as the potential target, tamoxifen was used to treat monoclonal antibody-Pgp in leukemia cell line K562, it was confirmed that tamoxifen competed with the antibody for the binding [37]. Another target confirmed using the same technique was MRP1 in human cervical carcinoma cell line HeLa [38]. Results ascertain that tamoxifen interacts with major MDR markers Pgp, MRP1 and LRP, and this in turn affects how antitumor drugs bind to transporter proteins, consequently inhibits MDR mechanism [37]. It is worth mentioning that this kind of interaction might lead to a decrease in intracellular concentration of tamoxifen and thereof lower its ER mediated activities. Thus, cells over expressing transporter proteins may show poor prognosis to tamoxifen use. This should not be mistaken as tamoxifen being effluxed out of tumor cells but rather as binding to Pgp, MRP1 or LRP and not to tamoxifen primary target [39] (Fig. 8).

4 Tamoxifen resistance and its clinical implication

Tamoxifen outstanding clinical outcomes have been devaluated by the fact that most of the patients with metastasis and about 40% of patients on adjuvant tamoxifen relapse [40]. Relapse occurs due to tamoxifen de novo and acquired resistance; this created a crucial need to understand the mechanism of these types of resistance, this understanding can help developing strategies to overcome tamoxifen resistance.

Tamoxifen resistance has been related to multiple mechanisms, all of which leads to dysregulation of ER. Major mechanisms include modulation of ER signaling, activation of oncogenic signaling pathways such as PI3K/Akt/Mtor and interference with growth factor signaling, and transcription factors [41].

Since most of the actions of tamoxifen are mediated via ER, ER expression has been considered a prognostic marker for tamoxifen effectiveness and therefore loss of ER expression is associated with de novo drug resistance. This is further confirmed by failure of tamoxifen to treat ER/PgR negative tumors [42, 43]. Reports suggested that around 20% of patients may suffer from acquired resistance despite they responded to anastrozole or to fluvestrant suggesting a role for ER in growth regulation even in many tamoxifen-resistant cases [44, 45]. Gene mutations are another common mechanism of tamoxifen resistance, although there has been a number of mutations that are induced in vitro yet only very few of those mutations are observed in patients [46, 47]. Mutations involving Asp351 were confirmed to abolish both agonistic and antagonistic activity of 4-OHTAM [48, 49]. Another distinctive mutation is changing Lys303 to Arg; this mutation was detected in 33% of patients with breast hyperplasia. This mutation promoted co-activator binding even with low estrogen levels and led to disturbance in ER dependent cell growth and consecutively induced drug resistance [50]. Some of the common mutation in LBD residues was not detected in the primary tumors obtained prior to endocrine treatment. These data indicate a novel mechanism of acquired endocrine resistance in breast cancer. Further studies are needed to assess the frequency of such mutations among patients and explore ways to inhibit its activity and restore sensitivity to hormonal therapy [51]. In 2015, Jeselsohn et al. studied recurrent ESR1 mutations in patients with metastatic ER+ disease treated by endocrine therapies including aromatase inhibitors. These mutations appeared as a hotspot inside the LBD,
this was accompanied by partial resistance to tamoxifen and fluvestrant and increased metastatic capacity [52]. Samples taken from newly diagnosed metastatic and loco-regional recurrence of endocrine-treated breast cancer showed that hotspot ESR1 mutations could emerge after or during adjuvant endocrine therapy including single-agent TAM, as well as during neoadjuvant endocrine treatment of primary tumors. The occurrence of these mutations usually confers a poor prognosis. Studying these mutations is essential in dictating suitable endocrine therapies [53].

Some epigenetic mutations like hypermethylation of CpG island leads to transcriptional inactivation of the ER gene. Therefore, promoters methylation status has been used as an indication of tamoxifen resistance development [54].

Spalt-like transcription factor 2 (SALL2), a member of the Cys2His2-like fold group (C2H2) zinc finger transcription factor family plays an essential role in cell growth and tumor progression [55, 56]. The loss of SALL2 abolished serum deprivation-induced cell cycle arrest, whereas SALL2 overexpression suppressed cell growth [57]. These results led to the assumption that SALL2 is a tumor suppressor. A contradicting observation led to considering SALL2 as an oncogenic protein. Researchers concluded that SALL2 ability to induce or suppress tumor depends on the protein and cell environment and the dominant signaling pathways. Ye et al. reported that SALL2 was significantly downregulated during tamoxifen therapy, this led to independent ER tumor growth and it conferred ER resistant phenotype in ER + cancer cells. SALL2 activity could be restored using DNA methyltransferase (DNMT), therefore co-administration of tamoxifen and DNMT inhibitors like 5-Aza-20-deoxycytidine (5-Aza-dC), is a rational approach to re-sensitize patients tamoxifen resistant breast cancer patients [58].

Drug-metabolizing enzymes (DMEs) did not receive much attention despite their possible involvement in tamoxifen metabolic drug resistance. Cytochrome P450 enzymes mediate tamoxifen biotransformation, CYP3A4, CYP2B6, CYP2C9, CYP2C19 and CYP2D6 are the essential isoenzymes for this process. The hydroxylated metabolites, 4-OHTAM (2) endoxifen (3) showed higher growth inhibition potency than the parent drug [59]. Endoxifen, the most potent metabolite is produced via N-demethylation of tamoxifen to form N-desmethyltamoxifen (NDM) whereas CYP2D6 catalyzed 4-hydroxylation of NDM to form endoxifen. There are more than 300 variants of CYP2D6 reported, some of them interfere prominently with potential clinical outcome and resistance of tamoxifen [2, 60–65]. Some recent work aimed to develop tamoxifen analogues that can bypass CYP2D6 metabolism and use the esterase enzymes to produce the active metabolites to ensure equal clinical outcomes for all patients [66–68].

The prevalence of CYP2D6*4, CYP2D6*10, CYP3A5*3 and CYP2C19*2 allelic variants and their correlation with tamoxifen resistance was intensively investigated. Studies suggested that lower CYP2D6 activity is associated with diminished clinical results, increased risk of relapse and lowers the chances of free survival. As for CYP3A5 and CYP2C19, there was no significant correlation between CYP3A5*3 and the plasma concentrations of tamoxifen and its metabolites. Confirming with previous studies CYP2C19*2 was associated with better efficacy of tamoxifen. CYP3A5 and CYP2C19 plays a minor role compared to CYP2D6 enzyme [69–74]. Therefore, researchers concluded there is an association between CYP genotypes and clinical pathology of breast cancer. It was noted that there was significant association between intermediate and poor metabolizers of CYP2D6 and CYP2C19 and tamoxifen resistance [75]. The question of whether genetic polymorphisms of CYP2D6 can affect treatment outcome in patients with early postmenopausal breast cancer has been a matter of debate, the necessity of genotyping to guide tamoxifen therapy is even another ongoing argument [74, 76, 77]. Summary of various factors affecting tamoxifen resistance is summarized in Fig. 9.

5 Mechanisms of tamoxifen-induced apoptosis

Apoptosis plays a vital role in the modulation of neoplastic transformation of cells. Tamoxifen induces apoptosis via non-ER dependent mechanisms including inhibition of phospholipase C-, D- and protein kinase C-mediated pathways [78]. Tamoxifen additionally activates caspases 6, 7 and 9 [79]. Tamoxifen inhibits AKT and JNK activation yet it activates MAP kinase in glioma C6 cells [80]. Tamoxifen induces apoptosis in cells overexpressing Bcl2; it activates c-Jun N-terminal kinase (JNK), p38 kinase and phosphorylation of c-Jun, which in turn activates caspase 8 [81]. This mechanism was strictly limited to cells overexpressing Bcl2. Tamoxifen induced expression of p53 and p21(waf1/cip1), and stimulated G0-G1 phase cell cycle arrest [82]. Tamoxifen significantly affected cyclin D and cMyc expression and stimulated caspases 6, 7 and 9 [83]. Tamoxifen stimulates apoptosis and oxidative stress in a mechanism that involves elevation of mitochondria nitric oxide (NO) synthase. Tamoxifen suppresses mitochondrial respiration and decreases cytochrome c; this in turn stimulates mitochondrial lipid peroxidation. This eventually leads to the induction of tumor apoptosis [84, 85]. It is important to note that tamoxifen regulates intracellular cascade, leading to the suppression of mitochondrial respiration, which stimulates apoptosis. As it has been identified by recent studies, tamoxifen stimulated the activity of caspase-3
in MDA-MB-231 and BT-20 breast cancer cell lines. Besides, tamoxifen demonstrated a pro-apoptotic effect via stimulation of c-Jun NH2-terminal kinase 1 (JNK1) [86]. Tamoxifen inhibited phosphorylation of Akt (pAkt) and c-FLIP in cells isolated from cholangiocarcinoma tumor xenografts. Overexpression of c-FLIP is associated with inhibition of tamoxifen-induced apoptosis, whereas deletion of a calmodulin-binding domain on c-FLIP restored sensitivity to tamoxifen. In vitro, tamoxifen treatment led to stimulation of caspase 8 and 10 in Sk-ChA-1 cholangiocarcinoma cell line. Therefore, the pro-apoptotic effect of tamoxifen is partially dependent on the inhibition of FLIP expression and inhibition of pAkt and stimulation of caspase activity in vivo and in vitro, respectively [87]. Interestingly, cross-connection between the lower level of nasopharyngeal carcinoma-associated gene 6 (NGX6) and cancer cell resistance to tamoxifen has been observed. Tamoxifen activated Smad-2/3 and also upregulated expression of Smad-4 in NGX-6-expressing TRM-7 cells leading to inhibition of proliferation and induction of apoptosis [88]. Furthermore, tamoxifen-induced apoptosis was mediated via cancerous inhibitor of protein phosphatase 2A (CIP2A) / protein phosphatase 2A (PPA2) / phospho-Akt (p-Akt) cascade in MDA-MB-231, MDA-MB-468, MDA-MB-453 and SK-BR-3 cells [89]. In another study, tamoxifen demonstrated anti-cancer potential against MCF-7 breast cancer cell line via induction of apoptosis and inhibition of invasion. Tamoxifen intervention significantly reduced mitochondrial membrane potential (MMP) and the amount of ATP. Notably, the lower level of MMP represents a sign of early cell apoptosis. Moreover, once is the level of MMP decreased, apoptosis is irreversible [90]. The anti-cancer role of tamoxifen via up-regulation of factors associated with apoptosis was observed in MCF-7 cell line. More in-depth molecular analysis revealed numerous genes that were upregulated after incubation with tamoxifen. Most of them were categorized as pro-apoptotic or growth-related intermediates, that contributed to MAPK and/or Tp53 signaling pathways [91]. It is worth mentioning that several natural or synthetic drugs can modulate the pro-apoptotic efficacy of tamoxifen. Recent evidence suggested the beneficial aspect of co-treatment by atorvastatin combined with tamoxifen via elevated expression of pro-apoptotic factors (such as Bax and cytochrome C) in melanoma B16F10 cells compared to groups treated by only with tamoxifen or atorvastatin [92]. Similarly, naturally occurring flavonoids genistein combined with tamoxifen exerted a synergistic effect against HepG2 hepatocellular carcinoma cells via stimulation of pro-apoptotic processes [93]. Importantly, TAM combined with Caffeic acid phenethyl ester (CAPE) induced apoptosis through the activation of caspases and induction of DNA fragmentation in MCF-7 cells. Additionally, the synergistic effect of TAM and CAPE decreased the level of Bcl-2 and Beclin-1. In this regard, combination of TAM with CAPE may improve cytotoxic
efficacy of TAM and overcome possible resistance or lower toxicity [94]. Besides, natural occurring phytochemical thymoquinone increased tamoxifen induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cell lines [95]. Thymoquinone combined with TAM induced apoptosis via X-linked inhibitor of apoptosis protein (XIAP) degradation and Akt inhibition and subsequent activation of caspase-9 and PARP cleavage in breast cancer cells. Also, co-administration of TAM- thymoquinone induced apoptosis through the downregulation of Bcl-xL, Bcl-2 and higher expression of proapoptotic Bax, apoptosis-inducing factor (AIF), p27, and cytoC in breast cancer cells [96]. Moreover, the synergic pro-apoptotic effect of lauril gallate and tamoxifen was demonstrated in MCF-7 breast cancer cells. The combination of both substances resulted in a lower expression of Bcl-2 [97]. Additionally, co-treatment by tamoxifen and PI3K inhibitor LY294002 synergically induced expression of pro-apoptotic genes such as caspase-3 and -7, and p53 and p21, respectively. Further analysis revealed downregulation of pAkt and cyclin D1 after combined treatment. On the other hand, downregulation of anti-apoptotic genes, including Bcl-2 and survivin, was detected after LY294002 and tamoxifen co-treatment in MCF-7 cells [98]. In a recent study, tamoxifen-xanthene hybrid induced mitochondria-mediated apoptosis due to activation of PARP cleavage, upregulation of Bim gene expression and increase in Bax/Bcl2 ratio in MCF-7 cells [99]. Figure 10 represents an overview of mechanisms associated with tamoxifen-induced apoptosis.

Fig. 10  Mechanism of tamoxifen induced apoptosis
6 Tamoxifen-induced toxicity associated with oxidative stress and its prevention

Tamoxifen stimulates the generation of oxidative stress that can result in the damage of cellular structures [100]. Indeed, oxidative stress and inflammation contribute to the occurrence of tamoxifen-induced adverse event, hepatotoxicity [101]. However, current research offers several potential ways to prevent tamoxifen-induced toxicity associated with oxidative stress. Several reports suggested supplementation that exerted protective effects against hepatotoxicity induced by tamoxifen in vivo. Zinc inhibited oxidative stress and improved antioxidant activity through abrogation of tamoxifen-induced decrease of hepatic activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and increase of malondialdehyde (MDA) level in female Wistar rats [101]. Similarly, pretreatment with thymoquinone, a natural compound found in *Nigella sativa* seeds, normalized SOD activity and inhibited the rise of TNF-α in the model of tamoxifen-induced hepatotoxicity of female rats [102]. The co-treatment with lycopene increased the level of SOD, CAT, GPX, and GSH and downregulated the activity MDA as well [103]. Moreover, the combination of sodium butyrate and tamoxifen upregulated CAT, SOD, and GPX1 genes in rat bone marrow cells. Therefore, such combinatorial-treatment could be associated with the modulation of genotoxic effects of tamoxifen by reducing oxidative stress [104]. Besides, as was demonstrated in tamoxifen-treated female Sprague–Dawley rats, tamoxifen-phospholipid complex could ameliorate the hepatotoxicity of tamoxifen by diminishing toxicity markers such as lipid peroxidation or increasing the activity of antioxidant enzymes [105].

7 Oxidative stress implications for tamoxifen therapy and resistance

Mechanisms associated with oxidative stress contribute to tamoxifen-induced hepatotoxicity [101]. However, tamoxifen exerts anti-tumor efficacy independently of ER expression through various mechanisms, such as those associated with oxidative stress [90].

As discussed in previous chapters, the resistance to tamoxifen is mediated through various mechanisms. However, in the context of the interaction of tamoxifen and oxidative damage, the effect of oxidative stress on tamoxifen resistance must also be highlighted. Tumor cells promote the resistance to tamoxifen by elevated levels of ROS-protecting enzymes. Increased levels of anti-oxidant proteins were observed in tamoxifen-resistant MCF-7 cells when compared with non-resistant MCF-7 cells through Nrf2/anti-oxidant response element (ARE) activation [108]. The accumulation of tamoxifen and its metabolites increased oxidative stress in ER+ as well as ER- breast cancer cells resulting in cell death. However, as a respond, breast cancer cells increased the expression of Nrf2 that led to the activation of ARE and increased transcription of genes associated with anti-oxidation and multidrug resistance transporters, thus increasing the survival from oxidative damage induced by tamoxifen [109].

Nevertheless, the combinatorial treatment with tamoxifen and antioxidants could promote anti-cancer efficacy of tamoxifen as was demonstrated in the model of DMBA-induced mammary carcinogenesis in Sprague–Dawley rats in which an administration of tamoxifen in combination with riboflavin, niacin, and coenzyme Q10 restored lipid peroxide level and the activity of anti-oxidants accompanied by enhanced antitumor activity [110]. However, antioxidants can potentially decrease the efficacy of anti-cancer therapies. Cancer cells are often characterized by increased accumulation of vitamin C when compared to normal cells and may be therefore better protected against ROS-associated negative effects. The potential detrimental effects of vitamin C supplementation during cancer treatment is supported by the ability of vitamin C to dose-dependently protect cancer cells from tamoxifen-induced lipid peroxidation demonstrated in breast cancer model in vitro (MCF-7 cells) [111].

Based on the above discussion of available evidence, it can be assumed that the implication of the effects of oxidative stress on the efficacy or resistance to tamoxifen is not straightforward due to the effects of different circumstances obtained from available research results. Tumor cells can develop a resistance to tamoxifen as a result of promoted protection against ROS mediated via increased anti-oxidative enzymes. On the contrary, combinatorial treatment of tamoxifen and antioxidants was demonstrated to promote the anti-cancer activity of tamoxifen. However, this finding is not clear, as in another model of breast cancer, the accumulation of vitamin C by tumor cells led to their protection...
against the effects of tamoxifen. Therefore, we can conclude that the effects of oxidative stress/antioxidants on the efficacy of tamoxifen are not consistent in terms of resistance or efficacy of tamoxifen and require further investigation.

8 Conclusion

In conclusion, the consideration of the effects of tamoxifen on both tumor and non-tumor cells through oxidative stress involves the interaction of several mechanisms associated with the therapeutic success, acquired resistance, or toxicity of tamoxifen.

Authors’ contributions

Prof. LS reviewed the article and suggested the topic.
Assoc. Prof. NSA suggested the topic, contributed to writing, revising and editing the manuscript. She is the corresponding author.
Dr. MS contributed to writing the manuscript.
Prof. RN Dr. PK contributed to writing the manuscript.
Dr. AL contributed to writing the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

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