Tamoxifen as an antioxidant and cardioprotectant

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Abstract

Tamoxifen is widely used in the treatment of breast cancer and has been proposed as a prophylactic agent in this disease. Tamoxifen is an effective antioxidant and protects membranes and low-density lipoprotein (LDL) particles against oxidative damage. This antioxidant action is shared by endogenous and synthetic oestrogens. The ability of tamoxifen to protect LDL particles against the oxidative damage implicated in atherosclerosis may be an important factor in the reported cardioprotective action of tamoxifen in women being treated for breast cancer. In addition, tamoxifen has been found to act in a similar manner to oestrogens to lower plasma cholesterol levels. The cardioprotective action of tamoxifen may be a key factor in predicting the likely risk/benefit ratio for prophylactic tamoxifen treatment in otherwise healthy women, who have been calculated to be genetically predisposed to developing breast cancer. In the future, predisposition to breast cancer may be determined by genetic screening.

Evidence for a cardioprotective action for tamoxifen?

Introduction

Tamoxifen is widely used in the treatment of breast cancer and has now been proposed as a prophylactic agent for this disease [1,2]. Although hormonally related cancers such as breast cancer are the most common causes of mortality in women aged 40–60-years-old, in women over the age of 60 cardiovascular disease becomes the leading cause. Myocardial infarction is the main cause of death in women in this age group and far exceeds all other causes in the later years of life. This makes consideration of the long-term effects of tamoxifen therapy particularly important. Tamoxifen has been reported to have beneficial cardiovascular effects and the benefits of prophylactic tamoxifen therapy, including potential protection against coronary heart disease and osteoporosis, in addition to decreasing the risk of breast cancer should outweigh the risk of deleterious side-
effects. However, this high benefit/risk ratio remains to be demonstrated in large-scale trials. The evidence for the cardioprotective action of tamoxifen and its possible mechanisms will now be examined [3]. Retrospective studies on the two randomized arms of the Scottish adjuvant tamoxifen trial have revealed a significant decrease in the incidence of fatal myocardial infarction in breast cancer patients treated with tamoxifen [4]. Tamoxifen therapy for at least 5 years appears to have cardioprotective action in post-menopausal women similar to that of oestrogen. The Stockholm randomized trial of adjuvant tamoxifen therapy in post-menopausal women with early-stage breast cancer reported that even short-term tamoxifen therapy significantly decreased occurrence of coronary heart disease [5]. In addition, tamoxifen therapy for 5 years again produced a significant decrease in coronary heart disease, but had very little effect on thromboembolic disease [5].

**Effect on cholesterol and lipoprotein levels**

Treatment with tamoxifen as an adjuvant in a group of 123 women with stage-I and -II breast cancer produced a significant decrease in serum cholesterol levels (a known risk factor for coronary heart disease) compared with a control group of 81 women with stage-I and -II breast cancer who were not taking a hormonal treatment or supplement [6]. In this study decreases in serum cholesterol of more than 10 mg/dl were recorded in 73% of tamoxifen-treated patients compared with 35% of controls. Decreases in cholesterol of more than 40 mg/dl were found in 40% of tamoxifen-treated patients compared with 13% of controls. The age of the women and their initial serum cholesterol level were found to be important factors in the magnitude of the decrease in cholesterol levels observed. In a study on 45 post-menopausal Indian women with breast cancer, evaluation after 3 and 6 months showed significantly decreased levels of total cholesterol and low-density lipoprotein (LDL) cholesterol (another important risk factor) and increased levels of high-density lipoprotein (HDL) cholesterol, in patients receiving tamoxifen [7]. A study of 24 patients receiving chemohormonal therapy (tamoxifen alone or in combination with chemotherapy) reported average decreases in total serum cholesterol of 17% and in LDL cholesterol of 27% [8] and these potentially beneficial decreases in cholesterol were observed regardless of whether tamoxifen was administered alone or in combination with cytotoxic chemotherapy [8]. In the Wisconsin Tamoxifen Study, a randomized placebo-controlled double-blind study in disease-free women, tamoxifen treatment was found to lower total cholesterol levels by 26 mg/dl and LDL cholesterol levels by 20% after 3 months of treatment [9,10]. These changes were maintained throughout the 2 years of tamoxifen treatment, and women with greater baseline cholesterol levels showed greater decreases with tamoxifen treatment. Similarly the Royal Marsden Hospital preventative feasibility trial, again in disease-free women, reported a 15% decrease in serum cholesterol levels [11,12].

**Effect on fibrinogen and antithrombin III levels**

The Wisconsin Tamoxifen Study reported also that fibrinogen levels decreased by 52 mg/dl after 6 months and this may be associated with a decreased risk of arterial thrombosis [10,13]. A small decrease in antithrombin III levels were also found in some women. However, none of the subjects displayed a
clinically significant decrease in antithrombin III levels and this small decrease is unlikely to explain the small thrombophlebitis-promoting effect of tamoxifen. Indeed, in a study of post-menopausal women receiving long-term adjuvant therapy with tamoxifen, associated thromboembolism was rare [12,14]. Furthermore, the Royal Marsden Hospital pilot trial reported no adverse effect of tamoxifen on coagulation (fibrinogen/antithrombin III ratio) [11].

**Effect on lipoprotein(a) levels**

Tamoxifen has been reported to decrease the circulating levels of lipoprotein(a) in post-menopausal women and in breast cancer patients [15,16]. Similar findings have also been reported for oestrogen [15]. This could be of particular importance because lipoprotein(a) levels have been suggested to account for much of the previously unattributable risk for coronary heart disease [17]. Plasma levels of this particular lipoprotein are not influenced by the dietary changes that can lower many of the other risk factors for coronary heart disease. Lipoprotein(a) differs structurally from LDL only by an extra protein molecule, apolipoprotein(a), which resembles plasminogen and is linked to apolipoprotein B-100. The reasons for the extreme atherogenicity of lipoprotein(a) are not yet clear but following oxidative modification it may promote macrophage transformation to foam cells (even more effectively than oxidized LDL [18] — see below) and it may directly promote the growth of atherosclerotic plaques. The apolipoprotein(a) moiety may cause blood clots to persist because its molecular similarity to plasminogen could enable it to compete with, and thus prevent, the normal action of plasminogen [17].

**Effect on homocysteine levels**

Tamoxifen has been reported to decrease plasma levels of the amino acid homocysteine [19]. This may also contribute to its cardioprotective action because elevated plasma levels of homocysteine is a known risk factor for cardiovascular disease [20]. Premature vascular disease and thrombosis are associated with elevated homocysteine levels and it is of interest that homocysteine can induce tissue factor activity in endothelial cells [21]. In addition, homocysteine can induce oxidative damage to LDL [22], which is implicated in atherosclerosis (see below). The plasma homocysteine level is controlled partly by genetic factors [23]. Various inborn errors of homocysteine metabolism (homocystinuria) can cause elevation of plasma homocysteine and patients with this metabolic disorder frequently suffer from cardiovascular disease in early adolescence, or earlier [24]. Even moderately elevated homocysteine levels have been reported to be associated with an increased risk of premature cardiovascular disease in retrospective case control studies [20,25] and in prospective studies [26,27]. Folate and cobalamin deficiencies can also cause elevated plasma homocysteine levels. Oestrogen status may be another influencing factor and pre-menopausal women have lower plasma homocysteine levels than post-menopausal women and men [28]. Furthermore, pregnancy and oestrogen replacement therapy decrease plasma homocysteine levels [29,30]. In a study of 31 post-menopausal women with breast cancer the plasma homocysteine level was decreased by a mean value of 30% after 9–12 months of
tamoxifen treatment [19]. It appears that tamoxifen is acting like oestrogen to produce this beneficial effect on plasma homocysteine levels.

Tamoxifen appears to have significant beneficial effects on some of the risk factors for coronary heart disease. Similarly, post-menopausal oestrogen use is associated with a reduction in the incidence of coronary heart disease as well as in mortality from cardiovascular disease, but does not influence the risk of stroke [31]. Protection against cardiovascular disease could thus be a health benefit of both disease therapy and preventative treatment with tamoxifen.

**Contribution of antioxidant action to cardioprotection?**

**Inhibition of microsomal and liposomal lipid peroxidation**

Tamoxifen, its derivatives and 17\(\beta\)-oestradiol (structures shown in Fig. 1) are all good inhibitors of lipid peroxidation in microsomal and pre-formed liposomal systems [32–36]. 4-Hydroxytamoxifen was found to be a better inhibitor of microsomal lipid peroxidation in both the Fe(III)-ascorbate and Fe(III)-ADP/NADPH systems and of liposomal peroxidation, than tamoxifen, 3-hydroxytamoxifen (droloxifene) or 17\(\beta\)-oestradiol. Time-course studies showed that tamoxifen and related compounds and 17\(\beta\)-oestradiol (all at their IC\(_{50}\) concentration...
tions) inhibited microsomal and liposomal lipid peroxidation throughout the incubation period and there was no clear evidence of a lag period followed by an acceleration of peroxidation to the control rate. This suggests that these compounds are unlikely to be classical chain-breaking antioxidants, even though hydroxy groups with potentially donatable hydrogen atoms are present in many of these compounds (tamoxifen itself being an exception). It has been suggested that these compounds act in part or in whole by stabilizing membranes against peroxidation via a decrease in membrane fluidity [37,38]. Indeed, such an ability to decrease membrane fluidity has been demonstrated for tamoxifen and related compounds [39,40]. Tamoxifen and 4-hydroxytamoxifen have both been reported to be effective inhibitors of Fe(III)-dependent lipid peroxidation in rat cardiac microsomes [41]. Tamoxifen was also a good inhibitor of lipid peroxidation in liposomes prepared from the phospholipid obtained from rat liver microsomes [41]. The role of lipid peroxidation in cardiovascular injury [42,43] and the development of atherosclerosis is well documented [44–47]. Some of the cardioprotective effect of tamoxifen may therefore be related to its ability to inhibit membrane lipid peroxidation.

When introduced into liposomes during their preparation [37], tamoxifen inhibited lipid peroxidation to a greater extent than cholesterol (it cannot enter pre-formed liposomal membranes and thus has no effect in that system). 4-Hydroxytamoxifen and 17β-oestradiol were approximately equipotent and both were more effective than tamoxifen. These results indicate the superiority of tamoxifen and related compounds over the natural membrane component cholesterol. Cholesterol is vital for cell growth and is taken up as LDL cholesterol at an enhanced rate by rapidly proliferating cancer cells, including breast cancer cells, which increase their expression of the LDL receptor as they become hormone independent and their growth becomes more uncontrolled [48]. This may explain the association between low serum cholesterol levels and cancer [49]. The total-cholesterol- and LDL-cholesterol-lowering abilities of tamoxifen (see above) may thus contribute to its inhibition of breast cancer cell growth (by depriving the cells of cholesterol for growth) as well as contributing to its cardioprotective action.

Protection of LDL against oxidative damage

Tamoxifen and in particular 4-hydroxytamoxifen have been reported to protect isolated human LDL against oxidative modification [50]. This may be of importance because oxidative damage to LDL is considered to be an important stage in the development of atherosclerosis: it is a prerequisite for macrophage uptake and cellular accumulation of cholesterol [18,44]. Lipid peroxidation is thought to start in the polyunsaturated fatty acids of the phospholipids on the surface of LDL and then propagate to core lipids, resulting in modification of the cholesterol, phospholipids and the apolipoprotein B molecule, in addition to the polyunsaturated fatty acids [18,51,52]. In a study on the action of tamoxifen and related compounds on oxidative damage to LDL, isolated human LDL was stimulated to undergo lipid peroxidation by the addition of Cu(II) ions [50]. This is a widely used experimental system [44,47] that is relevant to events occurring within the atherosclerotic lesion [53]. 4-Hydroxytamoxifen was more effective as
an inhibitor of Cu(II) ion-dependent lipid peroxidation than tamoxifen and 17β-oestradiol and also prevented peroxidation-induced modifications in the surface charge of the LDL, whereas tamoxifen did not. These alterations in the surface charge of LDL are associated with its recognition and uptake by macrophages in atherosclerotic lesions [44]. This action of the 4-hydroxy metabolite of tamoxifen could be particularly important to the observed beneficial cardiovascular effects of tamoxifen therapy. The lack of effectiveness of tamoxifen itself in preventing alteration of the surface charge of LDL may be because it is a much less effective inhibitor of lipid peroxidation than 4-hydroxytamoxifen. Tamoxifen, over the concentration range tested (5–30 μM), maximally inhibited LDL peroxidation by only approximately 50% compared with approximately 90% achieved by 4-hydroxytamoxifen. In the presence of tamoxifen, therefore, it is likely that sufficient aldehydic breakdown products of lipid peroxidation such as malondialdehyde and 4-hydroxynonenal are produced to modify the ε-amino groups of the lysine residues of the apolipoprotein B molecule and thus the surface charge of LDL [50]. It would also be of interest to determine whether tamoxifen can protect against homocysteine-induced lipid peroxidation of LDL [22] (see above) in addition to its ability to lower plasma homocysteine levels [19]. Tamoxifen and its derivatives are highly lipophilic compounds that are likely to accumulate in the atheromal plaques associated with the damaged arterial wall to achieve the protective concentrations reported [50]. Tamoxifen (and 4-hydroxytamoxifen) may stabilize LDL against lipid peroxidation by interactions between their hydrophobic rings and the polyunsaturated residues of the phospholipid layer of LDL, this suggestion is supported by the inhibition of lipid peroxidation arising from similar interactions in liposomal membranes [3,39,54].

**Comparison with oestrogen**

Although tamoxifen is often described as an anti-oestrogen anti-cancer drug it can also be a partial or full oestrogen agonist depending on the target organ and tissue. Tamoxifen actually shows considerable structural similarity to sterols including oestrogen (see Fig. 1) and the similar cardioprotective actions observed for both tamoxifen and oestrogens suggest that tamoxifen acts as an oestrogen-mimicking cardioprotectant. Oestrogen is recognized to have a protective effect against coronary atherosclerosis [31] and its ability to protect LDL against oxidative damage [50,55,56] could contribute to the cardiovascular benefits observed on oestrogen administration in post-menopausal women [31], independently of a favourable alteration of the plasma lipid profile. Studies in rabbits and monkeys have shown the anti-atherogenic effect of hormonal replacement to be independent of variations in lipid profiles. Female cholesterol-fed rabbits treated with oestradiol for 33 weeks developed less atheroma in arterial tissue than controls, even though no differences in total cholesterol or lipoproteins were observed [57]. In oophorectomized female monkeys on an atherogenic diet, hormonal replacement with 17β-oestradiol, either alone or with cyclical progesterone for a period of 30 months, significantly decreased the development of coronary artery atherosclerosis independently of lipid profile changes [58].

17β-Oestradiol inhibits the peroxidation of isolated human LDL *in vitro* [50,55,56] and has now been shown to inhibit the oxidation of LDL in post-
menopausal women. This was measured by the time of the onset of LDL oxidation (i.e. the lag time in the presence of Cu(II) ions [47]) in LDL isolated from women treated with 17β-oestradiol [59]. Acute intra-arterial infusion of 17β-oestradiol increased serum oestradiol levels from typical post-menopausal levels to physiological concentrations for reproductive-aged women at mid-cycle [60] and significantly prolonged the lag time of the LDL compared with baseline levels, indicating a decrease in susceptibility to oxidation. Transdermal patch administration of oestradiol for 3 weeks again increased serum levels of oestradiol and significantly prolonged the lag time but there were no significant changes in lipid profiles compared with baseline values. One month after discontinuation of treatment the LDL lag time had returned to baseline levels [59], suggesting that prolonged hormonal replacement therapy would be required to maintain the cardioprotective benefits. However, oestrogen hormonal replacement therapy has been indicated to carry similar risks of endometrial cancer to tamoxifen prophylaxis and requires careful monitoring [61]. It is clear that clinical investigations similar to the studies on 17β-oestradiol are required to explore the in vivo effects of tamoxifen on LDL oxidation. Clinical evidence for the inhibition of LDL oxidation by tamoxifen would be an important addition to the debate on the use of tamoxifen to prevent breast cancer in post-menopausal women [3,62].

**Tamoxifen as a cardioprotective agent: the future**

The justification for the routine use of tamoxifen as a protective agent against breast cancer in healthy women could depend not only on its effectiveness as a

![Cardioprotective actions of tamoxifen](image-url)
prophylactic agent but also on its other beneficial health effects such as its possible cardioprotective action. The results of the large prophylactic tamoxifen trials are thus awaited with interest to see if significant cardioprotection is observed. An overview of the cardioprotective actions of tamoxifen is shown in Fig. 2. Alternatives to tamoxifen for breast cancer treatment (and possibly in the future prevention) are being developed including the tamoxifen derivative droloxifene [63] and the pure oestrogen antagonists (derived from 17β-oestradiol) such as ICI 164 384 and ICI 182 780 (structures shown in Fig. 3). Droloxifene has several advantages over tamoxifen, including a shorter terminal elimination half-life, lower accumulation, improved drug tolerance and decreased occurrence of resistant cancer cells and a decreased risk of endometrial cancer, and is currently being used in a trial with women with advanced breast cancer [63]. Furthermore, droloxifene has an effective antioxidant action [33]. ICI 182 780 is being investigated for use with breast cancer patients [64] particularly those who have suffered a relapse following onset of resistance to tamoxifen therapy [65]. However, it is
important to note that the cardioprotective plasma cholesterol-lowering action of tamoxifen arises from its partial oestrogen antagonist action. Pure anti-oestrogens may still achieve a cardioprotective action similar to that of tamoxifen providing they possess a good antioxidant ability because it is likely that protection of LDL against oxidative damage is an important component of the cardioprotective action of tamoxifen. It is of considerable interest, therefore, that ICI 164 384 has been reported to be a good inhibitor of lipid peroxidation in both microsomal and liposomal systems [36]. The order of effectiveness in the microsomal system was 4-hydroxytamoxifen > 17β-oestradiol > tamoxifen > ICI 164 384 and in the liposomal system 4-hydroxytamoxifen > 17β-oestradiol > ICI 164 384 > tamoxifen. This indicates that this pure anti-oestrogen was overall of comparable effectiveness with tamoxifen, although not as effective as 4-hydroxytamoxifen and 17β-oestradiol. The ability of these new pure oestrogen antagonists to protect LDL against oxidative damage also requires urgent investigation to provide a more relevant indicator of possible antioxidant cardioprotective action.

References
49. Manninger, F. (1983) Cancer Res. 43, 2503s–2507s