Tamoxifen Acutely Relaxes Coronary Arteries by an Endothelium-, Nitric Oxide-, and Estrogen Receptor-Dependent Mechanism

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ABSTRACT
In epidemiological studies tamoxifen has been associated with a reduction in the incidence of fatal myocardial infarction in women. However, the effects of tamoxifen on coronary artery reactivity are unknown. We hypothesized that tamoxifen would relax precontracted isolated rabbit coronary arteries. Rings of coronary artery from adult male and nonpregnant female New Zealand White rabbits were suspended in organ baths containing Krebs’ solution; isometric tension was measured. Tamoxifen (0.1–100 µM) induced significant endothelium-dependent relaxation in precontracted rabbit coronary arteries. This relaxation was inhibited by Nω-nitro-L-arginine methyl ester and the estrogen receptor antagonist ICI 182,780. There was no significant effect on calcium concentration-dependent contraction curves. These data suggest that tamoxifen has beneficial effects on coronary reactivity that could, at least in part, account for the reduction in risk of fatal myocardial infarction in women taking tamoxifen.

Tamoxifen belongs to a group of structurally diverse compounds called selective estrogen receptor modulators (SERMs) that are able to interact with the estrogen receptor, but are distinguished from estrogen by an ability to act as either an estrogen agonist or antagonist, depending on the target tissue and hormonal milieu. Early recognition of the tissue-specific activities of tamoxifen, the first SERM, led to the development of new SERMs (raloxifene) that have greater tissue selectivity. The estrogen antagonistic properties of tamoxifen are useful in the treatment and control of breast cancer (Early Breast Cancer Trials’ Collaborative Group, 1992).

Epidemiological evidence suggests that tamoxifen confers a beneficial effect on the cardiovascular system, similar to estrogen. Tamoxifen is associated with a reduction in the incidence of fatal myocardial infarction in women (McDonald and Stewart, 1991; Early Breast Cancer Trials’ Collaborative Group, 1992), as well as suppressing diet-induced formation of lipid lesions in mouse aorta (Grainger et al., 1995). Tamoxifen appears to share with estrogen the beneficial effects on plasma lipids and lipoproteins (Rossner and Wallgren, 1984; Love et al., 1994; Stevenson et al., 1994). Estrogen relaxes coronary arteries acutely and chronically, both in vitro and in vivo (Jiang et al., 1991, 1992b; Mugge et al., 1993; Chester et al., 1995; Sudhir et al., 1995), and the mechanisms involved in this relaxation may contribute to the cardioprotective effect of estrogen. Recently, we have demonstrated that the SERM raloxifene shares this relaxant effect on coronary arteries (Figtree et al., 1999), the significance of which is currently being investigated in postmenopausal women in an international multicenter clinical trial called RUTH (Raloxifene Use in The Heart) (Barrett-Connor et al., 1998). The effects of tamoxifen on coronary vascular reactivity are unknown, therefore we investigated the effects of tamoxifen on rabbit coronary arteries in vitro.

Materials and Methods

Animals and Tissues. Adult male or nonpregnant female New Zealand White rabbits (2.5–3 kg) were sacrificed by an overdose of pentobarbitone (60 mg kg⁻¹) and heparin (150 units kg⁻¹). The heart was removed and epicardial coronary arteries were dissected free of connective tissue. Arterial rings were prepared and in some rings the endothelium was removed by gentle rubbing with a wooden probe. Each ring (2–3 mm in length) was suspended horizontally between two stainless steel parallel hooks for the measurement of isometric tension in individual organ baths containing 10 ml of modified Krebs’ solution at 37°C, bubbled with 95% O₂ and 5% CO₂. The composition of modified Krebs’ solution was as follows: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM K₂PO₄, and 11.1 mM glucose. Coronary arterial rings with or without endothelium from male and female rabbits were allowed to stabilize for 90 min under a resting tension of 1 g (9.8 mN) before being contracted. Preparations were exposed to maximally effective concentrations of a con-
tractile agonist (K⁺, 30 mM) to ensure stabilization of the rings. The agonist was then removed and the ring re-equilibrated. The presence of endothelium was verified by observing the relaxation response to acetylcholine.

**Effect of Tamoxifen on Precontracted Coronary Arteries.** Coronary arterial rings with and without endothelium were contracted with K⁺ (30 mM). Increasing concentrations of the tamoxifen (0.1–100 μM) were added at half-log increments at the plateau of the previous response. The response at each concentration of tamoxifen was measured. Simultaneous time-matched control curves were constructed using an equivalent volume of solvent as that used to dissolve tamoxifen.

**Effect of N°-Nitro-L-arginine Methyl Ester (L-NAME) on Tamoxifen-Induced Relaxation.** L-NAME is an inhibitor of nitric oxide (NO) synthesis from L-arginine in vascular endothelial cells (Rees et al., 1990). L-NAME (0.1 mM) was added to coronary arterial rings with endothelium 20 min before they were contracted with K⁺ (30 mM). This concentration of L-NAME has been demonstrated to completely inhibit nitric-oxide synthase in rabbit aorta (Rees et al., 1989). Concentration-dependent responses to tamoxifen (1–100 μM) were then measured.

**Effect of Barium Chloride on Tamoxifen-Induced Relaxation.** To examine the possible role of potassium conductance on tamoxifen-induced coronary relaxation, barium chloride, a nonspecific inhibitor of potassium channels (Quayle et al., 1988), was added to coronary arterial rings without endothelium 20 min before their contraction with K⁺ (30 mM). Concentration-dependent responses to tamoxifen (1–100 μM) were then measured.

**Estrogen-Receptor Dependence of the Relaxing Response to Tamoxifen.** To examine the possible role of the classical estrogen receptor in mediating tamoxifen-induced relaxation, rings with endothelium were incubated in the specific estrogen receptor antagonist ICI 182,780 (Wakeling et al., 1991) (10 μM) for 20 min before precontraction in K⁺ (30 mM). This concentration of ICI 182,780 was shown in single-cell studies to have antiestrogen receptor antagonism. Measurements of the responses to increasing concentrations of tamoxifen were then repeated.

**Effect of Tamoxifen on Calcium Concentration Responses in Rabbit Coronary Arteries.** Rabbit coronary arterial rings with or without endothelium were incubated in calcium-free solution containing 0.5 mM EGTA for 10 min. The calcium concentration-dependent contraction curves were then performed in high K⁺ depolarization medium (100 mM). Rings were readjusted in modified Krebs for 20 min and then incubated in calcium-free solution containing EGTA (0.5 mM) for a further 10 min. Subsequently, rings were incubated with tamoxifen (10 μM). The calcium concentration-dependent contraction curves were then repeated.

**Drugs.** The following drugs were used: tamoxifen (Sigma, Poole, Dorset, UK), L-NAME (Sigma), barium chloride (Sigma), and ICI 182,780 (Tocris, Avonmouth, UK).

**Data Analysis.** All results are expressed as mean ± S.E. Relaxation is expressed as percentage relaxation of contraction induced by K⁺ (30 mM). The results were analyzed with ANOVA, and the Student-Newman-Keuls test was used for multiple comparisons. A probability level of less than .05 was considered significant. n indicates the number of animals.

**Results**

**Relaxing Effect of Tamoxifen on Precontracted Coronary Arteries.** K⁺ (30 mM) induced significant contraction, comparable in rings with or without endothelium (Fig. 1). Tamoxifen induced significant concentration-dependent relaxation of contracted rings compared with time-matched controls (Fig. 2). Relaxation to tamoxifen in rings with no endothelium was significantly less than in rings with endothelium (Fig. 2). There were no significant differences in relaxation between arteries from male or female rabbits in rings with endothelium (Fig. 4a).

**Effect of L-NAME on Tamoxifen-Induced Relaxation.** Incubation with L-NAME (30 μM) had no significant effect on contraction response to K⁺ (30 mM) (Fig. 1). However, L-NAME significantly inhibited tamoxifen-induced relaxation in rabbit coronary arterial rings with endothelium (Fig. 3). The inhibitory effect of L-NAME was not significantly different in rings from male versus female rabbits (Fig. 4b).

**Effect of ICI 182,780 on Tamoxifen-Induced Relaxation.** Incubation in ICI 182,780 (10 μM) had no significant effect on contraction response to K⁺ (30 mM) (Fig. 1). However, ICI 182,780 had no significant effect on relaxation induced by tamoxifen in rings with endothelium (Fig. 3). The inhibitory effect of ICI 182 780 was not significantly different in rings from male versus female rabbits (Fig. 4b).

**Effect of Barium Chloride on Tamoxifen-Induced Relaxation.** The nonspecific inhibitor of potassium channels barium chloride (3 μM) had no significant effect on contraction response to K⁺ (30 mM). It also had no effect on relaxation induced by tamoxifen (Fig. 3).
Calcium Antagonistic Effects of Tamoxifen in Rabbit Coronary Arterial Rings. Tamoxifen (10 μM) had no significant effect on the calcium concentration-dependent contraction curves in high K⁺ (100 μM) depolarization medium compared with control. Maximal contraction was also not affected (Fig. 5).

Discussion

We have demonstrated that tamoxifen induces significant relaxation in precontracted rabbit coronary arteries. This relaxation was shown to be, at least in part, dependent on the endothelium and was able to be inhibited by L-NAME and the estrogen receptor antagonist ICI 182,780. Tamoxifen had no significant effect on calcium concentration-dependent contraction curves in this preparation. The relaxation of endothelium-denuded rings most likely occurs by a direct effect of tamoxifen on the vascular myocyte. A similar relaxing effect of tamoxifen on vascular reactivity has recently been observed in cerebral arterial preparations contracted with oxyhemoglobin (Wickman and Vollrath, 2000).

An important aspect of this work is that we have demonstrated a relaxant effect of tamoxifen on coronary arterial ring preparations at concentrations at and above 3 μM. Serum concentrations of tamoxifen and its metabolites have been demonstrated to show a wide asymmetrical distribution in patients with breast cancer, depending on hepatic dysfunction, age, treatment duration, and the associated chemotherapy on tamoxifen pharmacokinetics (median, 0.4 μM; range, not detectable to 2 μM) (Peyrade et al., 1996). Thus, the concentrations of tamoxifen that induced coronary artery relaxation in the present study approximate to plasma levels found in patients treated with tamoxifen. The mechanisms of coronary relaxation by tamoxifen demonstrated in this study may contribute to the apparent reduction in risk of cardiovascular disease in postmenopausal women taking tamoxifen (McDonald and Stewart, 1991).

NO is synthesized in the endothelium from l-arginine by NO synthase (Palmer et al., 1988) and can diffuse rapidly to smooth muscle, causing relaxation via stimulation of soluble guanylate cyclase and a subsequent increase in cyclic GMP (Rapoport et al., 1983). By blocking NO synthesis with L-NAME (Rees et al., 1989) we were able to completely abolish the difference in relaxation to tamoxifen between rings with and without endothelium, implying that the endothelial contribution to tamoxifen-induced relaxation is dependent on NO.

In the present study, significant tamoxifen-induced relaxation occurred in rings denuded of endothelium, or in which endothelial NO production was inhibited, suggesting that tamoxifen exerts a direct relaxant effect on vascular smooth muscle (Figs. 2 and 3). The relative contribution of this direct effect to tamoxifen-induced coronary artery relaxation ap-

Fig. 3. Graph showing effects of removal of endothelium (no endo; n = 8) and, in rings with endothelium, effects of incubation in L-NAME (n = 11), in ICI 182,780 (n = 9), and in BaCl (n = 6) on tamoxifen-induced relaxation compared with control rings with endothelium (with endo; n = 16). *, indicates significant difference in comparison with relaxation in rings with endothelium (*P < .05; **P < .01; ***P < .005).

Fig. 4. a, graph showing tamoxifen-induced relaxation in rings with endothelium from male (♂) versus female (♀) rabbits. Data are expressed as percentage relaxation of contraction induced by K⁺ (mean ± S.E.). There was no significant difference in relaxation (n = 8). b, effect of L-NAME on tamoxifen-induced relaxation in coronary arterial rings from male (n = 7) versus female (n = 5) rabbits. Data are expressed as percentage relaxation of contraction induced by K⁺ (mean ± S.E.). There was no significant difference in relaxation (P > .05). c, effect of ICI 182,780 on tamoxifen-induced relaxation in coronary arterial rings from male (n = 6) versus female (n = 3) rabbits. Data are expressed as percentage relaxation of contraction induced by K⁺ (mean ± S.E.). There was no significant difference in relaxation (P > .05).
pears to increase, compared with the endothelial component, with increasing concentrations of tamoxifen (Fig. 3). The lack of effect of tamoxifen on the calcium concentration-dependent con traction curve (Fig. 5) suggests that this direct smooth muscle effect is independent of calcium channel blockade. This is dissimilar to both 17β-estradiol (Jiang et al., 1992a; Han et al., 1995), whose actions on the vascular myocyte have been shown to involve an inhibitory action on voltage-gated calcium channels, and raloxifene in which the direct effect on vascular smooth muscle has also been demonstrated to involve calcium channels (Figtree et al., 1999). It also differs from results using whole-cell patch-clamp studies in vascular smooth muscle cells. Song et al. (1996) demonstrated that tamoxifen reduced current through L-type Ca2+ channels, with an ID50 of 2 × 10−7 M in A7r5 cells. This effect may therefore involve other ion channels not examined in the present study. Recent evidence that tamoxifen activates a large-conductance chloride channel in cultured endothelial cells, supports this possibility (Li et al., 2000). The insignificant effect of nonspecific potassium channel blockade with barium chloride suggests that modulation of potassium channel opening is not an important mechanism of endothelium-independent relaxation to tamoxifen.

We have demonstrated that the endothelium-dependent relaxation to tamoxifen is partially inhibited by the specific estrogen receptor antagonist ICI 182,780, suggesting that the response involves the estrogen receptor (Wakeling et al., 1991). This apparent estrogen receptor dependence has also been demonstrated for raloxifene-induced relaxation in the same model (Figtree et al., 1999). However, conditions in this rabbit model are significantly different to that in the human. In vitro and in vivo concentrations do differ with regard to potency. This model does not include effects of other circulating hormones, blood volume, or coronary circulation. Arteries from female rabbits are continually exposed to the high levels of estrogen of the estrous stage of the cycle. This may result in a different acute response to sex steroids in vitro compared with humans, in whom estrogen levels vary during the menstrual cycle, and are low after menopause.

We have shown that tamoxifen is able to act both via the endothelium and directly on the vascular smooth muscle to induce relaxation of epicardial coronary arterial rings from both male and female animals. The former mechanism involves interaction with the estrogen receptor at the level of the endothelium and stimulation of NO. With the increasing use of tamoxifen as an adjuvant treatment for the treatment of breast cancer patients, and the fall in mortality from breast cancer, the effect of this treatment on the risk of cardiovascular disease, the greatest cause of mortality in women in Western countries, becomes more important (Beral et al., 1995). This highlights the importance of the beneficial effects of tamoxifen on coronary artery vasomotion by a mechanism that may contribute to an overall beneficial effect of tamoxifen in protecting against cardiovascular disease (McDonald and Stewart, 1991; Early Breast Cancer Trials Collaborative Group, 1992).

References


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