Nitric Oxide Stimulatory and Endothelial Protective Effects of Idoxifene, a Selective Estrogen Receptor Modulator, in the Splanchnic Artery of the Ovariectomized Rat

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ABSTRACT

Estrogen is known to stimulate endothelial nitric oxide production and attenuate endothelial dysfunction after ischemia and reperfusion. However, estrogen therapy increases the risk of breast and endometrial cancer. The present study was designed to determine whether idoxifene, a selective estrogen receptor modulator without adverse effects on reproductive organs, may stimulate nitric oxide release and protect endothelial function. In U-46619 precontracted superior mesenteric arterial (SMA) segments isolated from ovariectomized rats, idoxifene and 17β-estradiol resulted in a comparable dose-dependent vasorelaxation (maximal relaxation: 75.3 ± 4.9 and 71 ± 4.7%, respectively). Treatment of the rings with Nω-nitro-L-arginine methyl ester completely blocked idoxifene- and 17β-estradiol-induced vasorelaxation. In vitro incubation of SMA rings with TNFα significantly reduced vasorelaxation to an endothelium-dependent vasodilator, acetylcholine (maximal relaxation: 73 ± 3.7 versus 95 ± 2.9% pre-TNFα, P < .01). Idoxifene, but surprisingly not 17β-estradiol, prevented TNFα-induced endothelial dysfunction (maximal relaxation: 86 ± 2.6% in idoxifene-treated rings and 77 ± 5.1% in 17β-estradiol-treated rings). In vivo ischemia and reperfusion resulted in significant endothelial dysfunction as evidenced by decreased vasorelaxation to acetylcholine (maximal relaxation: 48 ± 5.5 versus 92 ± 3.9% in normal SMA rings), but a normal reperfusion response to an endothelium-independent vasodilator, acidified NaNO2 (95 ± 3.2%). Treatment with idoxifene at either 1 or 2 mg/kg/day, or 17β-estradiol at 1 mg/kg/day for 4 days significantly preserved endothelial function (P < .01 versus vehicle). Taken together, these results demonstrate that idoxifene is an endothelium-dependent vasodilator and exerts significant endothelial protective effects against TNFα- and ischemia-reperfusion-induced endothelial injury. These results suggest that selective estrogen receptor modulators have therapeutic potential in diseases where endothelial dysfunction plays an important role.

Endothelial dysfunction manifested as decreased bioactive nitric oxide (NO) levels is one of the most common pathological changes occurring in various cardiovascular diseases such as ischemia/reperfusion, heart failure, and atherosclerosis (Harrison, 1994; Angus, 1996). Endothelial dysfunction contributes significantly to subsequent functional and cellular injury in a variety of pathological pathways. It disturbs the balance between vasorelaxation and vasoconstriction, and thus may promote vasoconstriction and contribute to the “no reflow phenomena” seen after ischemia and reperfusion. Endothelial dysfunction may also exacerbate tissue injury indirectly by increasing platelet-leukocyte-endothelium interactions. Therapeutic strategies aimed at improving endothelial function have been shown to markedly retard the development of atherosclerosis and attenuate vascular and tissue injury associated with ischemia/reperfusion (Lefer et al., 1991).

Estrogen replacement therapy after menopause has been shown to reduce the morbidity and mortality of cardiovascular diseases (Barrett-Connor and Bush, 1991). The mechanisms by which estrogen evokes its protective effect are not fully understood. Previous studies have suggested that estrogen may exert its cardiovascular protection by improving plasma lipid profiles (Blum and Cannon, 1998). However, this change accounts for only 25 to 50% of the protective effect of estrogen against cardiovascular diseases (Gruchow et al., 1988). Accumulating evidence now indicates that estrogen has a direct effect on the vascular endothelium with

ABBREVIATIONS: NO, nitric oxide; NOS, nitric-oxide synthase; SERM, selective estrogen receptor modulator; Ovx, ovariectomy; TNFα, tumor necrosis factor-α; DMSO, dimethyl sulfoxide; SMA, superior mesenteric artery; K-H, Krebs-Henseleit buffer solution; ACh, acetylcholine; L-NAME, Nω-nitro-L-arginine methyl ester; SI/R, splanchnic ischemia/reperfusion.
increased NO bioactivity, which may contribute significantly to its cardiovascular protective effects (Kauser and Rubanyi, 1997; Miller, 1999). Estrogen increases NO production via a traditional genomic pathway that up-regulates endothelial nitric-oxide synthase (NOS)-III gene expression, as well as a novel nongenomic pathway that directly enhances NOS activity (Kauser and Rubanyi, 1997; Kim et al., 1999). Estrogen may also increase bioactive NO levels via inhibition of superoxide production (Arnal et al., 1996; Dubey et al., 1999), thus preventing NO from destruction by reactive oxygen species.

Despite the apparent beneficial effects of estrogen in preventing cardiovascular diseases, it is estimated that <10% of women who might benefit from this therapy are actually taking it (Harris et al., 1990). The major reasons for this are fear of estrogen-induced breast and uterine cancer (Judd et al., 1983). The search for more acceptable and safer postmenopausal hormone replacement therapies has led to the evaluation of compounds known as selective estrogen receptor modulators (SERMs). Previous pharmacological studies have demonstrated that idoxifene, a tamoxifen derivative, is a novel SERM that has estrogen agonism on one or more desired target tissues such as bone and liver, and estrogen antagonism and/or minimal estrogen agonism in reproductive tissues such as the breast or uterus (Nuttall et al., 1998; Mitlak and Cohen, 1999). However, whether idoxifene may stimulate NO release and protect the endothelium from injury caused by pathological factors such as cytokines and ischemia and reperfusion has not been elucidated. Accordingly, the aims of the present study were 1) to determine the vasodilatory characteristics of idoxifene in vascular segments isolated from ovariectomized (Ovx) rats, and 2) to evaluate the effect of idoxifene on endothelial dysfunction caused by in vitro incubation with TNFα and in vivo exposure to ischemia and reperfusion.

**Experimental Procedures**

**Materials.** Idoxifene (pyrrolidino-4-iodotamoxifen) was synthesized by SmithKline Beecham Pharmaceuticals (King of Prussia, PA) and 17β-estradiol was purchased from Sigma Chemical Co. (St. Louis, MO). For in vitro study use, a 10 mM stock solution of idoxifene or 17β-estradiol was made with dimethyl sulfoxide (DMSO). For in vivo administration, idoxifene or 17β-estradiol was made as a suspension with 0.1 M lactate and 248 mM dextrose in saline at a concentration specified below (Treinen et al., 1998). All other compounds were purchased from Sigma Chemical Co., unless otherwise stated. Adult female Sprague-Dawley rats (300-350 g body weight) were obtained from ACE Animals Inc. (Boyertown, PA) and an ovariectomy or sham ovariectomy was performed on animals. The experiments were performed in adherence to National Institutes of Health Guidelines on the Care and Use of Laboratory Animals and were approved by the Thomas Jefferson University Committee on Animal Care and Use.

**Comparison of Vasorelaxation Activity of Estrogen and Idoxifene in Precontracted Superior Mesenteric Artery (SMA) Segments Isolated from Ovx Rats.** Two weeks after ovariectomy, rats were randomized to receive one of the following daily treatments in vivo for 4 days: 1) vehicle (0.1 M lactate, 248 mM dextrose in saline) plus 3 μM idoxifene, or TNFα plus 3 μM 17β-estradiol. Two hours after incubation, K-H buffer containing drugs was completely replaced with normal K-H buffer. After another three complete washouts, the rings were again checked for endothelium-dependent (ACh) and endothelium-independent (acidified NaN3) vasorelaxation. The ACh-induced vasorelaxation after TNFα incubation was compared with that before TNFα incubation.

**Comparison of the Effects of In Vivo Treatment with Estrogen and Idoxifene on Endothelial Dysfunction Induced by In Vitro Exposure of SMA Segments to TNFα.** SMA rings from normal Ovx rats were prepared the same way as described above. After a stable contraction to 50 nM U-46619 was established, ACh, an endothelium-dependent vasodilator, was added to the bath in cumulative concentrations of 10^-9 to 10^-5 M. After the cumulative response stabilized, the rings were washed, allowed to equilibrate to baseline, and randomly assigned to one of the following three groups: TNFα (10 ng/ml) plus vehicle (7 μl of DMSO), TNFα plus 3 μM idoxifene, or TNFα plus 3 μM 17β-estradiol. Two hours after incubation, K-H buffer containing drugs was completely replaced with normal K-H buffer. After another three complete washouts, the rings were again checked for endothelium-dependent (ACh) and endothelium-independent (acidified NaN3) vasorelaxation. The ACh-induced vasorelaxation after TNFα incubation was compared with that before TNFα incubation.

**Comparison of the Effects of In Vivo Treatment with Estrogen and Idoxifene on Ischemia/Reperfusion-Induced Endothelial Dysfunction.** Two weeks after ovariectomy, rats were randomly assigned to receive one of the following daily treatments in vivo for 4 days: 1) vehicle (0.1 M lactate, 248 mM dextrose in saline) (Treinen et al., 1998); 2) idoxifene suspension (0.5, 1, or 2 mg/kg/day, oral gavage); or 3) 17β-estradiol suspension (1 mg/kg/day, oral gavage). Idoxifene dose was chosen from a previous study demonstrating that administration of idoxifene at a dose range of 0.5 to 2.5 mg/kg/day effectively prevents bone loss and lowers cholesterol level in ovariectomized rats without producing unwanted estrogenic effects on the endometrium, suggesting that idoxifene acts as an SERM at this dose range (Nuttall et al., 1998). On day 4 after the start of treatment (60 min after the last drug administration), rats were anesthetized with sodium pentobarbital (50 mg/kg) i.p. injection. After a midline laparotomy was performed, the celiac and superior mesenteric arteries were isolated from surrounding connective tissues near their aortic origins. Splanchnic ischemia/reperfusion (SI/R) was induced by total occlusion of the SMA and the celiac artery with nontraumatic clamps. After 60 min of ischemia, the occlusive clamps were removed. The rats were then observed for an additional 180 min. Sham SI/R rats were subjected to all the surgical procedures performed on SI/R shock rats, including isolation of the SMA and celiac arteries, except that these arteries were not occluded.
After termination of the in vivo observation, rats were sacrificed via an overdose of pentobarbital (100 mg/kg). The superior mesenteric artery was removed and SMA rings were prepared as described above. After equilibration, the rings were contracted with 50 nM U-46619. Once a stable contraction was obtained, ACh, an endothelium-dependent vasodilator, was added to the bath in cumulative concentrations of $10^{-9}$ to $10^{-5}$ M to determine endothelial function. After the cumulative response stabilized, the rings were washed and allowed to equilibrate to baseline. The procedure was then repeated with an endothelium-independent vasodilator, acidified NaNNO$_2$ ($10^{-8}$ to $10^{-4}$ M) to determine smooth muscle function. NaNNO$_2$ was prepared by dissolving the compound in 0.1 N HCl and titrating it to pH 2.0. Titrating distilled water to pH 2.0 and adding aliquots to the bath did not produce any vasorelaxation.

**Comparison of the Effects of In Vivo Treatment with Estrogen and Idoxifene on Plasma NO Concentration in Ovx Rats.**

To determine the effect of idoxifene or estrogen treatment on plasma NO change in Ovx rats, 0.2 ml of arterial blood was withdrawn from sham-ovariectomized rats or Ovx rats treated with vehicle, idoxifene (1 mg/kg/day), or 17β-estradiol (1 mg/kg/day). Blood was centrifuged at 600g for 10 min (Beckman GS15R) and plasma NO concentration was determined using a chemiluminescence detector. To each 0.2 ml of plasma, 0.4 ml of ice-cold 100% alcohol was added. The plasma-alcohol mixture was placed in ice for 30 min and then centrifuged at 250g for 5 min. The supernatant (protein free) was used to measure the concentration of NO and its end product (i.e., nitrate and nitrite) (NOx = NO + NO$_3$ + NO$_2$) by using the vanadium reduction method (Ma et al., 1997). Briefly, 50 μl of sample was injected into a water-jacketed, oxygen-free purge vessel containing 5 ml of 0.1 M vanadium (III) chloride (Aldrich, Milwaukee, WI) in 2 N HCl (Sigma Chemical Co.). Acidic vanadium (III) at a temperature above 80°C reduced both nitrite and nitrate to NO, which was then quantified by a chemiluminescence detector (SIEVERS 270B nitric oxide analyzer; SIEVERS, Boulder, CO) after reaction with ozone. Signals from the detector were collected and analyzed using a MacLab data acquisition system. A standard curve was obtained using the area under the curve after each injection of 50 μl of 0, 12.5, 25, 50, 75, and 100 μM sodium nitrate.

**Plasma Estradiol Assay.** Plasma estradiol was determined by radioimmunoassay using a double antibody estradiol procedure following the manufacturer’s manual (Diagnostic Products Corporation, Los Angeles, CA).

**Statistical Analysis.** All values in the text and figures were presented as mean ± S.E. of n independent experiments. All data were subjected to ANOVA followed by the Bonferroni correction for post hoc t tests. Probabilities of P ≤ .05 were considered to be statistically significant.

**Results**

Vasodilator Characteristic of Estrogen and Idoxifene in Precontracted SMA. In U-46619-precontracted SMA rings, cumulative addition of DMSO at the same volume as that of idoxifene or estrogen resulted in a maximal vasorelaxation of $17 \pm 1.3\%$ (n = 14 rings from five rats). The DMSO-induced relaxation was endothelium independent because addition of L-NAME had no effect. This vehicle-induced, nonspecific vasorelaxation was subtracted when calculating idoxifene-induced vasorelaxation. In endothelium intact SMA rings, cumulative addition of 17β-estradiol from 1 nM to 10 μM resulted in a concentration-dependent vasorelaxation with an EC$_{50}$ of 59.8 nM (r = 0.96) and maximal vasorelaxation of 71 ± 4.7% (n = 15 rings from five rats, Fig. 1A). In another group of rings, cumulative addition of idoxifene from 1 nM to 10 μM also resulted in a concentration-dependent vasorelaxation with an EC$_{50}$ of 61.9 nM (r = 0.95) (n = 14 rings from five rats, Fig. 1B). Although the minimal effective concentration (0.1 μM) and the maximal vasorelax-
action (75.3 ± 4.9% at 10 μM) induced by idoxifene were comparable to that of 17β-estradiol, the relaxation response to idoxifene was significantly slower than that to 17β-estradiol (Fig. 2). Preincubation with L-NAME to inhibit endothelium nitric oxide synthase almost completely blocked vasorelaxation to idoxifene and 17β estradiol (Fig. 1, A and B). These results demonstrated that in rat SMA rings, both estrogen and idoxifene have an acute stimulatory effect on endothelial NO release and result in vascular relaxation in an endothelium-dependent manner.

**Effects of Estrogen or Idoxifene on TNFα-Induced Endothelial Dysfunction In Vitro.** Exposure of endothelial cells to TNFα induces a marked endothelial dysfunction as evidenced by decreased vasorelaxation to an endothelium-dependent vasodilator, ACh (Fig. 3), but an intact vasorelaxation to an endothelium-independent vasodilator, acidified NaNO2 (data not shown). Addition of 3 μM idoxifene with TNFα significantly preserved vasorelaxation to ACh (Fig. 3), suggesting that idoxifene exerted a protective effect against TNFα-induced endothelial dysfunction when applied in vitro. Surprisingly, although addition of 17β-estradiol in U-46619-precontracted SMA rings resulted in a vasorelaxation response comparable to that induced by idoxifene, addition of 17β-estradiol with TNFα only slightly improved (P > .05) ACh-induced vasorelaxation after TNFα incubation (Fig. 3).

**Effects of Estrogen or Idoxifene Treatment In Vivo on Plasma Estradiol Concentration.** Plasma estradiol concentration was significantly decreased in Ovx rats compared with sham-operated non-Ovx rats (0.05 ± 0.01 versus 0.19 ± 0.03 nM, P < .01, n = 10/group). Administration of 1 mg/kg/day 17β-estradiol for 4 days markedly increased plasma estradiol concentration (2.9 ± 0.08 nM, n = 9, P < .01 versus Ovx rats receiving vehicle). In contrast, administration of 1 mg/kg/day idoxifene had no significant effect on plasma estradiol concentration (0.08 ± 0.03 nM, n = 8).

![Fig. 2. Original tracings to demonstrate time course of relaxation in U-46619-contracted rat SMA rings to 17β-estradiol, idoxifene, and an endothelium-independent vasodilator, acidified NaNO2. The arrows indicate addition of U-46619 (50 nM); dots on top indicate addition of 17β-estradiol (10^−5–10^−9 M), idoxifene (10^−5–10^−9 M), and acidified NaNO2 (10^−4–10^−4 M).](image)

![Fig. 3. Effect of in vitro treatment with idoxifene and 17β-estradiol on TNFα-induced endothelial dysfunction in vitro. SMA rings were precontracted with 50 nM U-46619 and endothelium-dependent vasorelaxation to ACh (10^−6–10^−9 M) was performed before TNFα incubation and after 2 h of TNFα incubation (10 ng/ml) in the presence and absence of 3 μM idoxifene or 17β estradiol. **P < .01 versus vehicle. n = 14 rings/group from six or seven rats.](image)

These results suggested that the protective effects of idoxifene against ischemia/reperfusion-induced endothelial dysfunction described below were not achieved via increasing plasma estradiol concentration.

**Effects of Estrogen or Idoxifene Treatment In Vivo on Ischemia-Reperfusion-Induced Endothelial Dysfunction.** Endothelial dysfunction is one of the earliest pathological expressions occurring after organ ischemia and reperfusion. To clarify whether in vivo administration of idoxifene or estrogen may protect the endothelium from ischemia-reperfusion injury, we studied the effects of idoxifene or estrogen treatment on endothelium-dependent vasorelaxation in isolated SMA segments subjected to in vivo ischemia and reperfusion. Figure 4A summarizes the vasorelaxant responses of isolated SMA rings from rats after SI/R to increasing doses of an endothelial-dependent vasodilator, ACh, or to an endothelium-independent vasodilator, acidified NaNO2. SMA rings from sham SI/R rats exhibited complete vascular relaxation to both the endothelium-dependent (10^−5 M ACh) and the endothelium-independent vasodilators (10^−4 M NaNO2). In contrast, the concentration-response curve to ACh showed a significant shift to the right in the SMA rings from vehicle-treated SI/R rats. Treatment with low-dose idoxifene (i.e., 0.5 mg/kg/day) did not improve vasorelaxation responses of SMA rings to ACh. However, SMA rings from SI/R rats treated with two higher doses of idoxifene and with 17β-estradiol demonstrated a significant improvement in endothelium-dependent vasorelaxation. These results demonstrated that idoxifene and estrogen exerted significant protective effects on endothelial function after ischemia and reperfusion.

To determine whether SI/R may have altered the responsiveness of the vascular smooth muscle to NO, we investigated the vasorelaxant effect of acidified NaNO2 in SMA rings isolated from all seven groups. As summarized in Fig. 4B, acidified NaNO2 induced a concentration-dependent vascular relaxation, with full relaxation occurring at a NaNO2 concentration of 10^−4 M. There were no significant differences among any groups at any concentration of NaNO2 tested.

**Effects of Estrogen and Idoxifene Treatment In Vivo on Basal NO Release.** It has been recently reported that...
NO production by endothelial cells is significantly decreased in postmenopausal women and in ovariectomized animals, and that estrogen significantly enhances NO production from endothelial cells. To determine whether in vivo treatment with idoxifene also restores in vivo basal NO production in ovariectomized rats, we directly measured plasma NO concentrations. As illustrated in Fig. 5, plasma NO concentrations were significantly decreased in ovariectomized rats compared with sham-ovariectomized female rats (9.6 ± 1.6 versus 16.3 ± 1.4 μM, P < .01). In a dose-dependent manner, treatment with idoxifene restored plasma NO concentrations, which reached a plateau not significantly different from sham-operated rats at a dose of 1 mg/kg/day. Treatment with estrogen at 1 mg/kg/day also significantly restored NO production to a level comparable to that seen with 1 mg/kg/day idoxifene. This indicated that treatment with estrogen, as well as a novel SERM, idoxifene, significantly preserved in vivo basal NO production in ovariectomized rats.

**Discussion**

Experimental and clinical studies have provided ample evidence that estrogen exerts a significant antiatherosclerotic effect and reduces morbidity and mortality from cardiovascular diseases. The exact cellular mechanism remains unclear, but recent work from many investigators has suggested that up-regulation of endothelial NO production may significantly contribute to the cardiovascular protection exerted by estrogen (Blum and Cannon, 1998). The most probable mechanism of estrogen-induced up-regulation of endothelial NO production is the transcriptional stimulation of NOS-III gene expression (Kauser and Rubanyi, 1997). However, recent experiments have revealed that estrogen may increase levels of bioactive NO through alternative pathways. These include inhibition of cytokine-induced down-regulation of NOS-III gene expression, post-translational modification of NOS-III protein, and modulation of NO degrading systems (e.g., reactive oxygen radical generation and antioxidants) (Kauser and Rubanyi, 1997). More importantly, two recent studies have demonstrated that estrogen directly activates NOS-III and increases NO production from bovine aortic endothelial cells (Kim et al., 1999) and fetal lamb pulmonary artery endothelial cells (Chen et al., 1999) via a novel nongenomic pathway. This NOS-III activation effect of estrogen is rapid (<5 min) and independent of NOS-III protein level, is mediated by estrogen receptors localized in cell membrane caveolae rather than the classical nuclear receptors (Kim et al., 1999), and is calcium and extracellular signal-regulated kinase dependent (Chen et al., 1999).

Despite the apparent beneficial effects of estrogen in preventing cardiovascular diseases, it is estimated that <10% of women who might benefit from this therapy are actually taking it (Harris et al., 1990). The major reasons for this are fear of estrogen-induced breast and uterine cancer (Judd et al., 1983). Previous pharmacological studies have demonstrated that idoxifene, a tamoxifen derivative, is a novel SERM. Idoxifene has estrogen agonism on bone and liver tissues, and produces estrogen-like beneficial effects on plasma lipid profiles. However, idoxifene differs from estrogen in a tissue-specific manner. In human endometrial cells, where estrogen is a potent agonist through the estrogen response element, idoxifene has negligible agonist activity. In the uterus, idoxifene has a pharmacologically favorable profile, lacking agonist and therefore growth-promoting activity. Moreover, idoxifene has been demonstrated to block
estrogen-induced gene expression in endometrial cells (Nuttall et al., 1998; Mitlak and Cohen, 1999).

The present study has provided evidence that idoxifene may exert estrogen-like endothelial protective effects. For the first time, we have demonstrated that in normal vascular segments isolated from Ovx rats, idoxifene results in an acute, nitric oxide-dependent vasodilatation comparable to that exerted by estrogen in this same preparation. Similarly, it has recently been reported that raloxifene, another SERM, acutely relaxes rabbit coronary arteries in vitro with a dose-response curve comparable to that of idoxifene found in the present study (Figtree et al., 1999). Moreover, in isolated vascular segments from rabbit (Figtree et al., 1999) and human (Nechmad et al., 1998), estrogen- or raloxifene (10\(^{-9}\)–10\(^{-6}\) M)-induced vasorelaxation is blocked by estrogen receptor \(\alpha\) antagonist, such as ICI 182,780. These results suggest that SERMs, such as idoxifene and raloxifene, may exert an estrogen agonist effect in vascular endothelial cells and may result in endothelial nitric oxide synthase activation and vascular relaxation via nongenomic, mitogen-activated protein kinase-dependent mechanisms similar to those recently demonstrated for estrogen (Chen et al., 1999).

We have also directly demonstrated that in Ovx rats, basal NO production in vivo is markedly decreased compared with normal female rats. Treatment with idoxifene restored basal NO production in a dose-dependent manner with full NO recovery (\(P > .1\) versus sham-ovariectomized rats) at an idoxifene dose of 1 mg/kg/day. There is no significant difference between idoxifene and estrogen in their maximal effects on NO production. These results indicate that idoxifene significantly preserved in vivo basal NO production in ovariec-tomized rats. The exact mechanism by which idoxifene may exert its basal NO restoration effect cannot be determined by the present study. However, it is conceivable that this effect is likely achieved through a traditional genomic pathway that induces an up-regulation of the NOS-III gene because idoxifene was administered in vivo over a prolonged period and accumulated, rather than instantly released NO, was measured.

TNF\(\alpha\), a cytokine that has been demonstrated to be involved in tissue injury in a wide variety of cardiovascular diseases, has been shown to induce a significant down-regulation of NOS-III (Yoshizumi et al., 1993). In cultured endothelial cells, TNF\(\alpha\) has been found to result in destabilization of NOS-III mRNA, possibly by inducing a protein that can enhance degradation of mRNA, and thus reduce transcription of NOS-III (Alonso et al., 1997). In vivo infusion of lipopolysaccharide markedly inhibits endothelial NO production and ACh-induced vasorelaxation (Peters and Lewis, 1996). Moreover, a recent study has demonstrated that TNF\(\alpha\) generated from smooth muscle cells in response to interleukin-1\(\beta\) stimulation reduces NOS-III expression in a smooth muscle-endothelial cell coculture system (De Frutos et al., 1999). In the present study, we demonstrated that in vitro TNF\(\alpha\) incubation resulted in a significant endothelial dysfunction and addition of idoxifene with TNF\(\alpha\) markedly blocked the cytotoxic effect of TNF\(\alpha\) and preserved endothelial function. Surprisingly, although estrogen and idoxifene resulted in comparable endothelium-dependent vasorelaxation in vitro and restored basal NO production to a compa-

![Fig. 5. Effect of in vivo treatment with idoxifene or estrogen on plasma nitric oxide levels in Ovx rats. Control blood was obtained from sham surgical-operated female rats. **\(P < .01\) versus Ovx + vehicle. \(n = 10\) to 15 in each group. Ido-0.5, idoxifene treatment 0.5 mg/kg/day; Ido-1, idoxifene treatment 1 mg/kg/day; Ido-2, idoxifene treatment 2 mg/kg/day; Est-1, 17\(\beta\)-estradiol treatment 1 mg/kg/day.](https://jpet.aspetjournals.org/content/791)
Rable level when administered in vivo, coicion of estrogen with TNFα only insignificantly attenuated TNFα-induced endothelial dysfunction. The mechanism responsible for this discrepancy could not be answered directly by the present study. However, it is possible that an additional nonestrogen receptor-dependent effect, such as an antioxidant effect, is involved in idoxifene’s protection against TNFα-induced endothelial injury in vitro. In this connection, it has been recently reported that 2-hydroxyestradiol, an antioxidant metabolite of estradiol, but not estrone, significantly attenuates peroxidation of membrane phospholipids via a nonestrogen receptor-dependent mechanism (Dubey et al., 1999). It is therefore possible that estrogen, but not idoxifene, needs to be metabolized in vivo to produce an antioxidant effect. Further studies that will directly address this question are currently under investigation.

Functional integrity of the endothelium is crucial for the maintenance of normal vascular homeostasis. The loss of NO release may have significant pathophysiological significance in a variety of cardiovascular disorders such as ischemia and reperfusion, atherosclerosis and heart failure. First, decreased NO release may promote vasoconstriction, thus reducing organ blood flow and aggravating tissue ischemia. Second, loss of NO release may facilitate platelet aggregation and release of platelet mediators (e.g., thromboxane A2 and platelet-activating factor), which may exacerbate tissue injury. Third, because NO is a potent endogenous inhibitor of PMN chemotaxis, adherence, and activation, decreased NO release may promote PMN-associated endothelial and tissue damage during the reperfusion period. In the present study, 60 min of ischemia followed by reperfusion resulted in severe endothelial dysfunction in the superior mesenteric artery as evidenced by a decreased vasorelaxation response to an endothelium-dependent vasodilator, ACh. Treatment with idoxifene at 1 mg/kg/day resulted in an improved endothelium-dependent relaxation that was comparable to that seen with estrogen at the same dose. This result demonstrates that SERM treatment protects endothelium from ischemia-reperfusion injury, suggesting that SERMs may reduce tissue injury associated with cardiovascular diseases where endothelial dysfunction plays an important role.

In summary, we have demonstrated in the present study that idoxifene, a novel SERM, acutely stimulated endothelial NO production in vitro likely through a nongenomic pathway, restored basal NO production in vivo likely via a traditional genomic pathway and NOS gene up-regulation, preserved endothelial function in TNFα-incubated vascular segments in vitro, and attenuated endothelial dysfunction associated with ischemia and reperfusion. Since SERMs share the beneficial effects of estrogen in lipid metabolism and vascular endothelial function without adverse estrogenic effects on reproductive tissues, they may prove to be a superior option over estrogen for prevention and treatment of cardiovascular diseases.

References


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