

## THE VASORELAXANT AND ANTI-INFLAMMATORY ACTION OF RALOXIFENE IN RAT AORTA IS PARTIALLY MEDIATED BY ESTROGEN RECEPTOR ALPHA

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Selective estrogen-receptor modulators (SERM) have the potential to retain most of the beneficial effects of estrogen while avoiding most of its adverse effects. Raloxifene is a SERM approved for the prevention and treatment of postmenopausal osteoporosis, and is also known to relax different arterial and venous vessels with unclear mechanisms. We investigated the vascular effects of raloxifene using a dual approach. First, acute vasomotor effects were tested on aortic rings from estradiol-replaced ovariectomized (OVX) rats to avoid fluctuations in circulating hormones. Second, experiments were performed in isolated rat aortic smooth muscle cells (SMC) to test potential anti-inflammatory effects. Raloxifene (0.1 pM-0.1 μM) induced acute vasorelaxation of precontracted aortic rings through endothelium- and NO-dependent, prostanoid-independent mechanisms. At higher concentrations, the magnitude of the relaxant response sharply increased up to 70% and was detected even in endothelium-denuded vessels, suggesting a dual mode of action. To determine if the vasorelaxant effect was retained under conditions of systemic inflammation, aortic rings were isolated from rats treated with 0.1 and 1 mg/kg LPS 4 h before sacrifice. Raloxifene at the concentrations relevant in vivo (below 0.1 μM) was still able to relax aortic tissues from rats treated with low-dose, but not high-dose LPS. To reproduce inflammatory conditions in isolated cells, aortic SMC were stimulated with a mixture of cytokines/LPS for 24 h. After this time, iNOS synthesis became detectable by Western blotting in untreated SMC but was significantly reduced by incubation with 0.1-1 μM raloxifene. Similarly, raloxifene treatment significantly decreased cytokine-driven nitrite accumulation at 1 μM only (p<0.05). The down-regulation of iNOS function was not due to drug toxicity and was mediated by ERα because this effect was abolished by pretreatment with the novel selective ERα antagonist MPP. By contrast, preincubation of aortic rings with MPP had no effect on the rapid vascular relaxation evoked by raloxifene. ERα protein expression in isolated SMC was reduced by cytokines/LPS by more than 70% but was partially restored on treatment with increasing concentrations of raloxifene through autologous, ERα-mediated pathways. In conclusion, raloxifene prevented cytokine-induced iNOS activation in isolated aortic SMC and relaxed aortic tissues of E<sub>2</sub>-replaced OVX rats possibly through different mechanisms. These effects were raised at concentrations that may be relevant in vivo and involved at least in part ERα activation.