Editorial

Unravelling the cardioprotective mechanism of action of estrogens

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See article by Xu et al. [1] (pages 836–844) in this issue.

Cardiovascular diseases are the major cause of morbidity and mortality in the developed world, and are increasing in significance in the developing world. Elucidation of the mechanisms involved in preventing the development of coronary artery disease and protecting the myocardium against the deleterious consequences of myocardial ischaemia therefore stays an important research goal. The sex hormone estrogen has received increased attention for its ability to exert cardioprotective effects against atherosclerosis, but it has become clear that estrogen also exerts a direct protective effect against ischaemia/reperfusion injury on the myocardium. In this edition of *Cardiovascular Research* Xu et al. [1] provide evidence for decreased TNFα production during ischaemia/reperfusion in the mechanism of estrogen-mediated cardiac protection.

There is an abundance of scientific evidence for the protective effect of estrogen against atherosclerosis, such as short-term vasodilating effects as well as long-term vascular protective and anti-atherosclerotic effects [2]. Epidemiological evidence shows that pre-menopausal women have a reduced risk for mortality from cardiovascular diseases [3] and that women are at a lower risk for the development of heart failure [4] and have enhanced cognitive function and reduced neurodegeneration associated with Alzheimer’s disease and stroke [5]. Further proof for the protective effect of female gender is the fact that post-menopausal women have a similar or even increased risk for cardiovascular disease compared to men [3] and have an increased risk for adverse outcome after myocardial infarction and acute coronary syndromes, despite similar treatment with thrombolysis and percutaneous interventions [6]. It was therefore postulated that estrogen replacement would be beneficial in preventing cardiovascular diseases in post-menopausal females. However, clinical trials designed to investigate the effects of estrogen replacement therapy in secondary prevention were surprising—in the Heart and Estrogen/Progestin Replacement Study (HERS) no overall cardiovascular benefit was found, and in fact an increase in coronary heart disease during the first year was observed [7]. In the Women’s Health Initiative (WHI) study estrogen/progestin replacement was stopped because an increased cardiovascular risk occurred [8]. This does not invalidate the significance of estrogen for cardiovascular protection, but probably tells us that we still have to elucidate the exact pharmacological means of attaining benefit for our patients.

The protective effect of female gender noticed in the epidemiological observations has mainly been attributed to an estrogen mediated decrease in the incidence of atherosclerosis. New evidence suggests that estrogen may also mediate direct cardioprotective effects independent of its ability to prevent atherosclerosis, yet little is known about the mechanism of this protective effect. Already in the 1980s it was shown that there were gender differences in the myocardial response to ischaemia [9], and that estrogen was probably responsible for these differences [10]. More recently, in a study to investigate the effect of exercise on tolerance to ischaemia/reperfusion injury, Brown et al. [11] showed that sedentary female rats had significantly smaller infarct sizes than sedentary males (25±3% vs. 37±3%) confirming gender-related endogenous protection. Interestingly, exercise by running on a treadmill had a more pronounced protective effect in males than in females.

The ability of pharmacological administration of estrogen to confer direct myocardial protection has been investigated. Acute pharmacological treatment with estrogen has been
demonstrated to protect against ischaemia. Hale et al. [12] found that 10 μg 17 beta-estradiol given intravenously to male rabbits protected against 30 min regional ischaemia, reducing infarct size by 20%, and was effective in both males and females [13]. Infarct size decreased from 23±5% to 10±1% in females and from 31±5% to 16±4% in male rabbits. These results show the efficacy of estrogen protection in both sexes, but also again demonstrate the underlying difference in response to ischaemia between the sexes—inferior size in control female rabbits was 33% smaller compared to males, despite similar risk zones for ischaemia. Further evidence for an acute protective effect comes from observations in dogs, where acute intravenous administration of conjugated equine estrogen protected both male and female dogs from ventricular arrhythmias induced by ischaemia/reperfusion [14].

The cardioprotective effect of estrogen is further supported by the findings that ovariectomy caused a loss of cardioprotection, which was regained by chronic estrogen replacement therapy. Implantation of subcutaneous pellets with slow release of estrogen over 2 to 3 weeks resulted in less structural damage [15], smaller infarct sizes [11], as well as improved functional recovery [16,17] compared to untreated animals. Beer et al. [16] found that estrogen replacement improved developed pressure in treated ovariectomised rats to 114±9% of pre-ischaemic value, compared to 70±8% in ovariectomized animals without estrogen replacement and 89±4% in control female rats when subjected in vitro to 15 min global ischaemia and 30 min reperfusion. Aortic flow upon reperfusion was 65±9% in the estrogen treated ovariectomized group versus 29±9% in the untreated ovariectomized group in an isolated perfused working heart model used by Kolodgie et al. [17]. These authors found the cardioprotective effect of estrogen to be dependent on chronic administration as it could not be mimicked by acute administration 20 min before the ischaemic event. These experiments confirm a loss of gender-related cardiac protection against ischaemia/reperfusion and the ability to regain protection by estrogen replacement therapy.

Estrogen is a steroid hormone and mediates its actions through binding to estrogen receptors (ER), as reviewed recently [18]. Two estrogen receptor subtypes, ERα and ERβ, are located in both nuclear and membrane/cyttoplasmic pools. Estrogen receptors form homodimers (and to a lesser extent heterodimers) that mediate its effects. The binding of 17β-estradiol to estrogen receptors can have nuclear effects (i.e. effects on transcriptional regulation), but may also have direct, fast, non-nuclear effects. The nuclear effects result in the recruitment of coactivators and displacement of corepressors at DNA binding sites, resulting in the modulation of gene expression and protein expression. Nuclear effects are mediated by binding of the estrogen–nuclear receptor complex to estrogen response elements in the promoters of target genes, as well as an estrogen response element independent mechanism. In the latter case, estrogen binds to regulatory elements such as the activator protein 1 (AP-1), SP-1, or cyclic AMP response element sites that bind to transcription factors. Long-term effects of estrogen on the vasculature are well known and include increased expression of the genes for prostacyclin and nitric oxide synthase, rapid vascular endothelialisation after injury, inhibition of proliferation of vascular smooth muscle cells, and an anti-apoptotic action [19].

Membrane ER binding results in rapid, nongenomic actions and are mediated by several pathways, such as receptor tyrosine kinases and protein kinases including PI3K, Akt, mitogen-activated protein kinase (MAPK), Src, and protein kinase A and C, and by increasing the concentration of intracellular calcium [19]. The ERα receptor has been mainly implicated in membrane effects, but there is evidence for a similar mode of action for ERβ [19]. With regard to cardiovascular events, direct membrane signalling causes vasodilatation through nitric oxide release and opening of the calcium-activated potassium channels through a NO and cyclic GMP pathway [19]. Membrane activation of ERα plays a role in the preservation of endothelial cell structure and function due to activation of the anti-apoptotic p38α MAPK and inhibition of pro-apoptotic p38β MAPK, causing an upregulation of Elk-2 and phosphorylation of heat shock protein 27 [19]. The downstream cellular effects cause induction of angiogenesis and preservation of stress fibre formation and membrane integrity protection against apoptosis. Knowledge about the membrane/non-genomic effects of ERα is still scant, but it is interesting to note that it includes an inhibitory effect on platelets [19]. It is important to note that these two mechanisms are not necessarily totally independent, and that membrane-mediated signal transduction activation can result in both non-nuclear and nuclear actions. For example, neuroprotection is dependent on membrane mediated p42/p44 MAPK activation in some neuronal cell lines, but in others depends on both membrane and genomic activation [19].

The growing knowledge about estrogen action and signal transduction is important, as it has enabled the development of drugs with more selective actions, including the selective estrogen receptor modulators (SERMs), which have varying agonist or antagonist actions in different cell types. For example, tamoxifen has ER antagonistic effects in the breast and lowers total serum cholesterol and LDL, but has growth effects on the endometrium. Raloxifene is a SERM mainly used to preserve bone, and stimulates eNOS activity in endothelial cells, causing ERα-mediated vasorelaxation, but does not stimulate the endometrium. In a study in dogs, raloxifene had cardiac effects such as improving coronary perfusion, cardiac contractility and myocardial metabolism [20]. The future development of selective cardioprotective estrogens is thus more than a theoretical possibility.

The vascular protective effects of estrogen and their mechanisms are thus fairly well-established. What do we know regarding the mechanism of estrogen mediated direct
cardioprotection? Firstly, the question arises whether the estrogen effects are receptor mediated or whether they are related to a non-specific steroidal effect. In the study by Hale et al. [12], administration of 17β-estradiol elicited protection against ischaemia, whereas 17α-estradiol, which has no receptor mediated effects, was ineffective, leading to the conclusion that the protective effect of estrogen was indeed a receptor-mediated event. Studies from knockout mice have contributed to our understanding of the role of the estrogen receptor subtypes in protection against ischaemia/reperfusion damage. Gabel et al. [21] have demonstrated the importance of the beta estrogen receptor subtype. Only β-estrogen receptor knockout (βERKO), and not αERKO female mice, lost protection against ischaemia. It must be noted, however, that the ER-related protective effect demonstrated by these authors occurred under very specific circumstances. Protection against ischaemia was only demonstrated in female mice in a model of hypercontractility induced by isoproterenol infusion 1 min prior to global ischaemia. The relevance of these findings to cardioprotection is therefore not certain.

What are the molecular/cellular mechanisms of estrogen mediated protection? Gabel et al. [21] investigated the role of altered metabolism in estrogen receptor knockout mice. Gene profiling revealed that the expression of SPOT 14, which regulates expression of hypoxigenous genes such as ATP citrate lyase, fatty acid synthase, and sterol CoA desaturase, was decreased in βERKO females. Furthermore, wild-type males also showed significantly less expression of these genes. The possible contribution of altered metabolism to cardioprotection was further investigated by studying carbohydrate utilisation, and these authors convincingly showed that female hearts used significantly more carbohydrates than males. The cardioprotective effect of enhanced glucose oxidation during ischemia/reperfusion is well known, and this proposed mechanism seems plausible. Indeed, chronic treatment with 17 β-estradiol increased glucose oxidation during reperfusion in the absence of any effect on glycolysis [22].

In this issue of *Cardiovascular Research*, Xu et al. [1] produce compelling evidence for another mechanism of estrogen-mediated protection, namely a reduction in tumour necrosis factor-α (TNFα) production during ischaemia/reperfusion. In an isolated working heart model, hearts of ovariectomized rats treated with estrogen replacement were exposed to 25 min global ischaemia and 40 min reperfusion. Ischaemia/reperfusion caused a significant increase in TNFα levels in the coronary effluent as well left ventricle tissue of estrogen-deficient rat hearts and reduced functional recovery upon reperfusion. Estrogen replacement resulted in decreased TNFα levels in the coronary effluent and left ventricle tissue as well as a significant improvement in functional recovery, less apoptosis, and less LDH release during ischaemia/reperfusion. Evidence for a causal relationship between estrogen replacement therapy and the reduction in TNFα production was provided by the finding that a TNFα sequestrant, etanercept, improved functional recovery in the untreated animals, but had no effect on the treated animals. Furthermore, chronic estrogen replacement changed the expression of TNF receptors—expression of TNFR1 increased and TNFR2 decreased in treated compared to untreated animals.

TNFα has a controversial role in ischaemia/reperfusion damage. A damaging role for TNFα was suggested by findings that mice lacking TNFα were protected against ischaemia/reperfusion injury [23]. This is contradicted by findings that infarct size was increased in mice lacking one or both TNF receptors [24], suggesting that endogenous TNFα was protective. Yet other data showed no role for TNFα in ischaemia/reperfusion injury as infarct size in TNFα−/− mice was unchanged compared to control animals [25]. The ability of etanercept to counter the deleterious action of TNFα in ischaemia/reperfusion injury as reported by Xu et al. [1] has also been demonstrated in dogs [26] and provides supportive evidence that TNFα is indeed involved in the deleterious myocardial effects of ischaemia/reperfusion. This notion is further supported by a recent study from Wang et al. [27], in which female rat hearts displayed a diminished inflammatory response to ischaemia/reperfusion compared to male rats. mRNA expression and protein levels of TNFα, IL-1β, and IL-6 and activation of the p38 MAPK signal transduction pathway were decreased and were accompanied by improved indices of recovery following ischaemia. The work of Kurrelmeyer et al. [24] demonstrates another point relevant to the observations of Xu et al. [1]—infarct size was unchanged if either one of the two TNFα receptor subtypes was lacking—both receptors had to be absent to change the response to ischaemia/reperfusion. In view of these findings it is unlikely that the change in TNF receptor expression played a role in the findings by Xu et al. [1]. Considering everything, the data strongly indicate that estrogen may induce a diminished inflammatory state in the myocardium that is beneficial during ischaemia/reperfusion and in which TNFα plays an important role.

The study by Xu et al. [1] has made an important contribution towards our understanding of the cardioprotective effects of estrogen and raises more questions. Are these experimental findings also valid in vivo? Were these effects the result of activation of ERα or ERβ, or are both needed? What is the mechanism of estrogen-mediated reduced TNFα production, and which signal transduction pathways are involved? What is the relationship between the previously reported metabolic alteration of increased glucose oxidation during reperfusion (from the same group [22]) and the reduction in TNFα production? What is the spectrum of the anti-inflammatory action of estrogen and does it involve more than one cytokine? Does this protective effect of estrogen hold in senescence, and does chronic estrogen use (months or years vs. weeks) maintain these effects?

In conclusion, our knowledge about the mechanism of action of estrogen and its role in the cardiovascular system
is expanding and will hopefully extend into a clinical application in the future.

References


