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ssessing benefits and risks of estrogen and hormone replacement therapy in postmenopausal women has been a long story with conflicting results and major changes in paradigms.1,2 Observational studies had consistently demonstrated a reduction in mortality and cardiovascular disease incidence in women on hormone replacement therapy compared to postmenopausal women not on replacement therapy. However, randomized trials have resulted in great disappointment because hormone replacement therapy for primary and secondary prevention of atherosclerotic disease were mostly associated with more risk (thromboembolism, malignancies, stroke) than benefit.1,3,4 However, the estrogen story has not been buried because of convincing preclinical data demonstrating a wide variety of cardiovasculoprotective action of estrogens.5 Among others, estrogen has major impact on the endothelium. The integrity of the endothelium effectively prevents atherosclerotic lesion formation and progression and has therefore come into the focus in vascular research. Estrogens trigger nitric oxide bioavailability through activation of endothelial nitric oxide synthase activity6 and reduce production of reactive oxygen species (Figure).7 Furthermore, estrogens accelerate endothelial cell regeneration after endothelial cell damage by mobilization of endothelial progenitor cells and activation of mature endothelial cells within the vessel wall.8-12 Nonendothelial cell–mediated effects of estrogens include effects on the inflammatory and immune system, the platelet cascade with prevention of arterial thrombosis (but promotion of venous thrombembolism) and beneficial effects on blood pressure (Figure).8 Additionally, novel clinical insights of estrogen replacement therapy have boosted and preserved the interest in estrogen-mediated vasculoprotection. The Women’s Health Initiative trial showed that treatment with conjugated equine estrogens alone could not reduce the risk of cardiovascular events compared with placebo treatment.13 In contrast to an elevated risk after treatment with conjugated equine estrogens plus medroxyprogesterone, the cardiovascular event rate was not increased in the single estrogen treatment group. Moreover, subgroup analyses showed that patients who received estrogens at <60 years with <10 years since menopause had a benefit from hormone replacement with estrogens alone, indicating that hormone therapy may only be effective in women in the early stages of menopause when risk factor burden is lower and atherosclerosis less severe.14 Taken together, as we continue to assemble the pieces of the puzzle of estrogen-mediated mechanisms, the full picture is only very slowly becoming visible.

In this issue of Circulation Research, Toutain et al add another important piece to the estrogen puzzle.15 In their study, they investigated the protective role of estrogen in a mouse model of skin ischemia mimicking clinical skin flap surgery. Estrogen deficiency after ovariectomy resulted in a severe necrosis of the skin flap, whereas physiological concentrations of estrogen prevented skin flap necrosis. Further analyses unraveled that estrogen-mediated activation of antiapoptotic pathways (Bel-2 protein expression) and structural and functional changes on the ultrastructural level (increase in mucinous layer important for the protection of the structural skin integrity, only mild endothelial cell damage with prevention of vascular leakage, remodeling of arteriolar anastomoses) account for the observed effects which finally prevent destruction of the vascular network. The protection of the structural components of the skin vasculature ultimately prevented the necrosis of the skin flaps by accelerated reperfusion conditions. The estrogen action was attributed to the estrogen receptor α (ERα) and independently of transforming growth factor-β and NO availability.

If we (boldly) assumed that we could draw a big picture from reports on estrogen effects on cardiovascular, skin, and other tissues, one may appreciate the following important aspects of the study by Toutain et al for further attention:

1. The timing of estrogen therapy and its associated effectiveness. The authors showed that estrogen was most effective when treatment was initiated 3 days before flap elevation, whereas initiation of therapy at the day of surgery had no effect on flap necrosis. Associations with the clinical trial results immediately come to mind concerning the timing of estrogen replacement therapy. It appears that estrogen therapy is required well in advance for protection at least before severe biological damage is present.
2. Higher doses of estrogen showed no further beneficial effect on flap necrosis. This is of interest because higher doses of estrogens are associated with more clinical adverse events. It appears that using estrogens in physiological doses seems to be adequate for effective vasculoprotection. In this context, the clinical studies predominantly used equine estrogens, which may have additionally influenced the outcome of the studies.

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3. Vascular network protection was supposedly endothelial cell–independent mediated by estrogen. It is well known that estrogens favor functional properties of endothelial cells. In this context, one would have expected a prevention of the necrotic skin flap, eg, by increases in angiogenesis. However, with elegant methodological approaches, Toutain et al\textsuperscript{15} were able to demonstrate that changes in flap recovery were independent of transforming growth factor-\textit{\beta} (as shown to be important in wound healing) but associated with a remodeling of arteriolar anastomoses within the flap. Surprisingly, proliferation of endothelial cells was increased in estrogen-deficient and substituted mice but extravasation and vascular leakage was enhanced in estrogen-deficient mice. This argues for an activation of endothelial cells but no severe endothelial cell damage, and, consequently, Toutain et al tentatively demonstrated that bone marrow–derived cells were not required for an effective flap recovery. Whether this holds true requires further investigations, including careful analysis of bone marrow reconstitution after transplantation, as well as a diligent evaluation of circulating cells. Moreover, regenerating cells could have been allocated from other places than the bone marrow, as lately shown in parabiosis models.\textsuperscript{16} In addition, the proposed mechanism of enhanced arteriogenesis and vascular network protection was not further investigated. Conversion of the quiescent endothelium into an activated monolayer is a key step in arteriogenesis and followed by adhesion of monocytes.\textsuperscript{17} The following invasion of monocytes is the prerequisite for effective arteriogenesis. Therefore, analysis of monocyte recruitment and the role of estrogens would have been of special interest because \textit{\beta}-estradiol, on the other hand, has been demonstrated to downregulate monocyte adhesion to the endothelium via downregulation of Rac1 GTPase.\textsuperscript{18}

4. Besides the vasculoprotective potential of estrogens attributable to the aforementioned mechanisms, one may be disconcerted because of these multiple features. Enhancement of cell survival and decreased apoptosis, improvement of vascularization, and enhanced angiogenesis are some of the key targets in anticancer treatment strategies. Is this the double-edged sword in estrogen therapy? Potential vasculoprotection that is outperformed by increase in malignancies as suggested by some clinical trials?

Again, it appears that the multiple facets of estrogen concerning its function and effect on target cells are more complex than we currently understand and definitely seek for further in-depth analyses. The novel findings of Toutain et al\textsuperscript{15} add valuable information to the estrogen puzzle and underline the importance to further investigate estrogens, its mechanism of action, and its clinical relevance in cardiovascular disease despite disappointing clinical results. Estrogen research is still worthwhile!

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