Ageing skin: oestrogen receptor β agonists offer an approach to change the outcome

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Abstract: Oestrogen (17β estradiol) and the dietary antioxidants resveratrol, genistein and S-equol, an isoflavone produced from the gut biotransformation of soy daidzein, are effective agents to reduce ageing in skin. It is widely held that these antioxidants scavenge free radicals to prevent skin damage. However, the evidence to date suggests that the primary mechanism of action of these antioxidants is to activate oestrogen receptor β (ERβ), which in turn enhances the expression of antioxidant enzymes and inhibits the expression of snail, a transcription factor that regulates keratinocyte cell proliferation and migration. Based on their selectivity, ERβ agents provide a treatment option for ageing skin without the potential safety issues associated with oestrogen therapy.

Key words: AP-1 – oestrogen receptor β – photoaging – resveratrol – S-equol
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Ageing skin: scope of the problem
‘Old age isn’t so bad when you consider the alternative’ (Maurice Chevalier-New York Times, 9 October 1960). Although ageing skin has minimal effects on long-term health, more than $50 billion a year is spent on skin products, which claim to prevent or reverse the ageing process. Although the skin is the largest organ in the body, it is one of the least studied and we know very little about the changes that occur with ageing. It is known that oestrogen plays a key role in ageing skin. In this viewpoint, we present the evidence for a selective oestrogen receptor β (ERβ) agonist as an agent to prevent the ageing process.

Reactive oxygen activates transcription factors AP-1 and snail in skin
Fine wrinkles, dry skin, hair loss and hyperpigmented spots are early indications of skin ageing. The accumulation of collagen breakdown products is a hallmark of ageing skin and results in a decrease in mechanical tension that leads to wrinkles (1). Age-related differences in the amount and structure of proteoglycans that bind to collagen also determine the mechanical properties of skin (2). There are multiple pathways (3) that can contribute to ageing skin, presenting a challenge for treatment. Both natural skin ageing and photoaging owing to exposure to the sun generate the superoxide radical (O₂·) that activates the transcription factor AP-1 (Fig. 1). This transcription factor consists of heterodimers of c-Jun and c-Fos. Phosphorylation of c-Jun by mitogen-activated protein kinases (MAP kinases) leads to increased AP-1 activity (4); retinoids, a mainstay for the treatment of photoageing, inhibit the activation of c-Jun. Activated AP-1 binds to the promoter region of the procollagen gene to inhibit its transcription, thus reducing the amount of collagen in skin. AP-1 also activates the matrix metalloproteinase genes MMP-1 (collagenase), MMP-3 (stromelysin) and MMP-9 (gelatinase), enzymes that degrade collagen (5). Another protein that is induced with UV irradiation through AP-1 is snail (6), a transcription factor that plays an important role in the epithelial-to-mesenchymal transition (EMT), promoting keratinocyte proliferation (7). Cysteine-rich protein-61 (CYR61) is another AP-1-activated protein that is elevated in the dermis of photoaged skin. Quan et al. (8) have shown that UV irradiation of human skin causes a sixfold increase in CYR61 mRNA and a fivefold decrease in collagen 1 protein.

Oestrogen protects skin from ageing
Although there is much to learn about the molecular pathways that affect skin, it is known that chronological ageing is associated with decreased skin thickness and increased elasticity and dryness in both men and women. Clinical studies have shown that oestrogen treatment improves skin quality in women and men (Table 1). Rittie et al. (12) evaluated the effects of topical oestrogen on procollagen expression in elderly (mean age 75 years) men and women. Volunteers were treated with 17β estradiol on sun-protected hip or on photodamaged skin obtained from the forearm or face. For the sun-protected hip, oestrogen treatment increased the amount of types 1 and 3 procollagen mRNA and protein in women; oestrogen also increased procollagen expression in men, though to a lesser extent than in women. Oestrogen treatment had no effect on procollagen expression in the photodamaged skin in the forearm or face in men or women. The lack of effect of oestrogen on photoaged skin could be related to the older age of the subjects, the short time of treatment or to irreversible genetic changes that occurred during the ageing process.

An interesting question raised by the Rittie et al. (12) study is whether treatment at an earlier age would have made a difference in the response to oestrogen on the photoaged skin of the 75-year-old subjects. In this regard, Wolff et al. (10) reported that postmenopausal women who had used hormone therapy (HT) continuously within 1 year of their last menstrual period had significantly lower wrinkle scores and skin rigidity, as measured by the durometer, than women who had never used HT. In another study (9), also with younger subjects, postmenopausal women aged 46–58 already using oral or transdermal oestrogen for at least 1 year were then treated with topical 0.01% micronized 17β estradiol and the dietary antioxidants resveratrol, genistein and S-equol, effective agents to reduce ageing in skin.

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Agents that activate ERβ prevent photoageing

Our understanding of the mechanism(s) of oestrogen’s action in skin has continued to evolve since this topic was reviewed in 2002 (13). Oestrogen’s action occurs through two oestrogen receptors, ERα and ERβ, that are related but distinct nuclear hormone receptors. The selectivity of an oestrogen receptor agonist for ERα or ERβ is determined by its relative binding affinity, differences in transcriptional activity and the tissue distribution of the receptors (14). Keratinocytes express mainly ERα with little or no ERβ (15,16). In contrast, dermal fibroblasts express both ERα and ERβ (17). Several lines of evidence indicate that ERβ is a key target for prevention of photoageing (18), wound healing (19) and skin tumour growth (20). Chang et al. (18) reported that the ERβ-selective agonists WAY-200070 and ERB-041 significantly reduced the levels of inflammatory markers of photoageing in human primary keratinocytes and fibroblasts in vitro: MMP-1, MMP-3, IL-6, IL-8, COX2 and TIMP-1 expressions were significantly reduced. In contrast, the ERα-selective agonist propyl pyrazole triphenol (PPT) had no effect on the UV-induced increase in these bio-markers. ICI 182,780, an oestrogen receptor antagonist for both ERα and ERβ, reversed the inhibition of MMP-1 expression by WAY-200070. Furthermore, WAY-200070 had no effect on the expression of MMP-13 (the mouse equivalent to human MMP-1) in the ERβ knockout mouse, indicating that ERβ is required for MMP-1 expression. To show that ERβ is protective against UV-induced damage, Chang et al. (18) exposed mouse skin to UV irradiation three times a week for 6 weeks. Importantly, ERB-041 was applied to the skin after irradiation, ruling out a free radical-scavenging mechanism. ERB-041 significantly reduced wrinkle formation at all concentrations tested, providing direct evidence for the role of ERβ in protecting skin from photodamage.

The isoflavones genistein and S-equol represent another class of ERβ-selective agonists (21) that have positive effects on skin. An isoflavone extract from soy cake and genistein, the major isoflavone present in soy, reduced UV-induced cell death in the human keratinocyte cell line HaCaT and attenuated the level of erythema when applied topically to mouse skin prior to irradiation (22). In humans, oral daily intake of 40 mg of soy isoflavone aglycones improved skin elasticity of middle age (late 30s and early 40s) Japanese women (23). In another study with Caucasian women (24), aged 45–65, consumption of a soy-based drink significantly reduced wrinkle depth in the crow’s foot area of the eye as compared to the control group; the test group had increased synthesis of type 1 procollagen. S-equol is not present in soy but is produced from daidzein (25). Being an ‘equol producer’, has many health benefits, including fewer menopausal symptoms and increased bone mineral density (26). Whether improved skin in subjects consuming soy isoflavones is attributable to S-equol needs to be determined using pure S-equol. However, animal studies show that topical racemic equol is photo- and photoprotective after UV irradiation (27–31).

Another antioxidant that protects human keratinocytes from UV damage and activates ERβ-mediated genes is resveratrol, a phytoalexin found in fruits, nuts and red wine. Ndiaye et al. (32) summarized the various animal and human studies that demonstrate its photoprotective and anti-proliferative properties. In animal studies with topical application, resveratrol inhibited the UV-mediated increases in skin oedema and infiltration of leucocytes (33). In cultured cells, resveratrol and oestrogen increased

### Table 1. Effects of topical and oral estrogen and isoflavones on skin

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal women</td>
<td>Topical 0.01% E2 vs. placebo</td>
<td>↑ Epidermal thickness</td>
<td>(9)</td>
</tr>
<tr>
<td>46–78 years (mean 51.3) HT for at least 1 year</td>
<td>Apply once a day to face 16 weeks treatment</td>
<td>↑Dermal thickness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cross-sectional analysis</td>
<td>↓Wrinkle score with HT</td>
<td>(10)</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>Topical 0.01% E2 vs. topical (4% genistein)</td>
<td>Applied to face once daily 24 weeks treatment</td>
<td>(11)</td>
</tr>
<tr>
<td>HT (mean age 55.7) vs. non HT (mean age 59.6)</td>
<td>Applied to face once daily 24 weeks treatment</td>
<td>E2 treatment was superior to isoflavone</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>Topical 0.01% E2 vs. placebo</td>
<td>Procollagen mRNA</td>
<td>(12)</td>
</tr>
<tr>
<td>45–55 years</td>
<td>Applied to face once daily 24 weeks treatment</td>
<td>Men: Women 2-fold</td>
<td></td>
</tr>
<tr>
<td>Men and postmenopausal women</td>
<td>Occlusion E2 1 week</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>75 years (mean)</td>
<td>Occlusion E2 1 week</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Sun protected skin (hip)</td>
<td>Occlusion E2 1 week</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Photodamaged skin (forearm)</td>
<td>Occlusion E2 1 week</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Photodamaged skin (face)</td>
<td>Cream E2 2 weeks</td>
<td>No effect</td>
<td></td>
</tr>
</tbody>
</table>

HT, Hormone Therapy; E2, 17β oestradiol.
cellular stress resistance to $H_2O_2$ by activating the mitochondrial enzyme Mn superoxide dismutase (SOD) (34). The oestrogen receptor antagonistICI 182780 prevented the increase in SOD. The conclusion that ER$\beta$ is involved in resveratrol’s action is based on the finding that diarylpropionitrile (DPN), a selective ER$\beta$ agonist, increased Mn SOD protein, whereas the ERz agonist PPT had no effect.

**Oestrogen receptor $\beta$ agonists prevent skin from ageing by multiple mechanisms**

Accepted dogma has the body’s own antioxidants and dietary vitamin E and C, as the front line in preventing photoageing. This conclusion is based in part on the finding that the amounts of these antioxidants are lower in photoaged skin of older individuals compared to younger subjects (35). Consistent with this finding, higher levels of hydrogen peroxide are present in skin fibroblasts from photoaged skin of the elderly compared to younger subjects (36). Although the dietary antioxidants genistein, resveratrol and S-equol scavage free radicals, we propose that their primary mechanism of action is by activation of antioxidant enzymes. SOD is an antioxidant enzyme that catalyses the breakdown of superoxide to hydrogen peroxide that is further metabolized by catalase and glutathione peroxidase to clear the body of damaging free radicals (Fig. 1).

The expression of antioxidant enzymes is regulated by NF-E2-related factor 2 (Nrf2) (37). This transcription factor is present as an inactive form in the cytoplasm of skin cells bound to Kelch-like ECH-associated protein 1 (Keap 1). Superoxide anion chemically modifies a cysteine residue in Keap 1 releasing Nrf2. As Nrf2 is now freed from its chaperone protein, it translocates to the cell nucleus to enhance gene expression. Resveratrol protects human keratinocytes from UV stress by increasing the levels of Nrf2 and by decreasing Keap 1 levels in the cytoplasm (38). In the nucleus, Nrf2 binds to antioxidant response elements within the promoters of the antioxidant enzyme genes to enhance transcription. Genistein, S-equol and resveratrol enhance the binding of Nrf2 to NAD (p)h:quinone oxidoreductase (QR), a phase 2 metabolizing enzyme (39). It is not known if ER$\beta$ is required for the increased binding of Nrf2. However, S-equol and resveratrol, but not genistein, also enhance the binding of ER$\beta$ to an oestrogen receptor element within the promoter region of QR.

Snail is a transcription factor that plays a key role in the EMT (40). In human skin keratinocytes, UV irradiation transiently induces snail by activation of AP-1 through MAP Kinase (6). In other cell types, snail is also activated through a TGF$\beta$ pathway that includes the transcription factor Smad 3 (41). However, in keratinocytes, snail is not activated by this mechanism (6). The genes activated by snail include several inflammatory response genes and MMP-9. With extended UV exposure, EMT leads to keratinocyte proliferation and migration. One gene that is down-regulated by snail and is a marker for EMT is E-cadherin, a tumour suppressor gene. Snail binds to the E-cadherin gene to inhibit its transcription. Oestrogen also represses E-cadherin expression by binding to ERz on the promoter of snail to enhance its expression. Park et al. (42) have shown in ovarian cancer cells that oestrogen promotes EMT through ERz. The ERz-specific agonist, PPT, reduces E-cadherin levels, similar to that for estradiol. In contrast, DPN, an ER$\beta$-selective compound, increases the expression of E-cadherin presumably by inhibiting the expression of snail. The role of ERz in skin to promote EMT and its impact on ageing skin needs further research. However, the increased risk of cancer in women on HT may be explained by oestrogen’s effect on EMT through ERz.

**Early treatment may be the panacea for ageing skin**

We propose that women who are treated with a topical ER$\beta$ agonist after menopause will have younger skin if they had been previously treated with HT prior to or during menopause, whereas the woman who waits until after menopause will show less skin improvement. This concept originated from the results of the Women’s Health Initiative where it was shown that women 50–79 years of age (mean 62.7) who received Premarin® (Pfizer Pharmaceuticals Inc., New York, NY, USA) (conjugated oestrogens) plus a progestin (medroxyprogesterone) had a greater risk of coronary heart disease, venous thrombosis, stroke and invasive breast cancer than the placebo group (43). With further analysis, it was shown that women who initiated HT closer to menopause had less cardiovascular risk than those who were 5 years past menopause (44). Based on these conclusions, Hodis and Mack (45) proposed a ‘window of opportunity’ for the prevention of coronary heart disease with HT. The hypothesis is that women need to initiate HT within 6 years of menopause and/or before 60 years of age to have a clinical benefit later in life. Similarly, Henderson and Brinton (46) have proposed a ‘critical window’ for oestrogen receptor agonists for cognition and Alzheimer’s disease.

Clinical trials are ongoing to test the ‘window hypotheses’. The Early versus Late Intervention Trial with Estradiol has randomized 643 women who had no pre-existing heart disease and were either <6 years or >10 years since menopause. The two groups were randomized to a placebo group and a group receiving oral oestrogen therapy (17$\beta$ estradiol). The primary end point is a change in carotid intimal thickness. The hypothesis being tested is that oestrogen will reduce the progression of atherosclerosis if started during or soon after menopause when the endothelium is healthy. Another ongoing trial is the Kronos Early Estrogen Prevention Study (KEEPS) that will determine whether early oestrogen therapy improves cognitive skills. The women are 42–58 years of age with their last menopause cycle occurring within 6 months to 3 years. The women are randomized into an oral oestrogen group (0.45 mg Premarin®), a transdermal patch (Climara®; Bayer Health Care Pharmaceuticals, Berlin, Germany) and a placebo group. In this study, there is a skin ancillary study (47) that will test the hypothesis that HT will have an impact on the rate of skin ageing. At the baseline measurements, there was no association between skin wrinkles and time since menopause. However, when the time since menopause in years was compared with skin rigidity, there was a significant improvement in forehead rigidity even at the baseline measurements, suggesting that there is a ‘window of opportunity’ for treating skin.

**Conclusions**

In this viewpoint, we propose that it is not the antioxidant properties of agents that protect skin from ageing, but their ability to activate ER$\beta$ that is responsible for their efficacy. This conclusion allows one to target specific biological pathways that are regulated by oestrogen receptors and opens new approaches that are specific
to skin. Because of the safety issues with HT, oestrogen therapy has limitations for the postmenopausal woman. A tissue-selective oestrogen offers one approach that may provide a safer alternative (48). As the breast and uterus contain ERα, a selective ERβ agonist may reduce the safety concerns associated with oestrogen therapy. The ongoing KEEPS trial may provide evidence that treatment for ageing skin should begin earlier in life before oestrogen levels begin to decline.

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Conflict of interest
As stated, RLJ and RJS have equity in Ausio Pharmaceuticals, LLC. We have no other conflict of interest.