

Estrogen Metabolites: Increasing Evidence for Their Role in Rheumatoid Arthritis and Systemic Lupus Erythematosus



In this issue of *The Journal* the research group chaired by Rainer Straub provides further data on the complex influence that estrogens (mainly 17 β -estradiol, E2) and their metabolites seem to play in autoimmune rheumatic diseases¹. In patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), their study investigated urinary concentrations of the downstream mitogenic 16-hydroxyestrone and 2-hydroxyestrone or naturally occurring antiestrogen.

As the investigators report, other studies in breast cancer research delineated that 16-hydroxyestrone is a mitogenic and proliferative endogenous hormone that covalently binds to the estrogen receptor leading to nuclear translocation^{2,3}. 16-Hydroxyestrone is converted from upstream estrone and E2, and because of this covalent linkage to the receptor, shows persistent biological responses consisting of mitogenic tumor growth-stimulation⁴. Further conversion products of estrone and E2 are the 2-hydroxylated estrogens such as 2-hydroxyestrone and 2-hydroxyestradiol. In contrast to 16-hydroxylated estrogens, the 2-hydroxylated forms inhibit growth-promoting effects of E2⁵.

Interestingly, the study shows that urinary concentrations of the 2-hydroxylated estrogens were 10 times lower in patients with RA and SLE than in healthy controls, whereas the ratio of 16-hydroxyestrone/2-hydroxyestrogens was 20 times higher in RA and SLE patients than in controls. They conclude that the magnitude of conversion to the mitogenic 16-hydroxyestrone is extremely upregulated in RA and SLE, which most likely contributes to the maintenance of the cellular proliferative state observed in these diseases.

Possible roles of hydroxyestrones

Of course, these observations are not totally new, and the authors review previous studies on the matter. Elevated serum concentrations of 16-hydroxyestrone have been described in patients with SLE⁶, suggesting that abnormal patterns of E2 metabolism may lead to increased estrogenic activity. A similar phenomenon was recently described in the synovial

fluids (SF) of patients with RA, where 16-hydroxyestrone/4-hydroxyestradiol were found to be significantly higher compared with control fluids^{7,8}. In these studies as well the molar ratio of free estrogens/free androgens was found to be significantly higher in RA SF (Figure 1).

Two important aspects must be considered. Total serum concentrations of E2 are not typically outside of physiologic ranges in patients of both sexes with RA and SLE, and the reported alterations in estrogen metabolism were again observed in both male and female patients, as recently reviewed⁹⁻¹¹.

Thus it is intriguing that sex may not influence the entire phenomenon and that the gonadal production of the sex hormones is not responsible for the observed metabolic results, since most of the measured metabolites are converted in the periphery, which is largely independent of sex. The phenomenon seems to be dependent only on the inflammatory state of the tissue and its presence in both RA and SLE

	RA	SLE
Tissue aromatase activity	↑	↑
Hydroxyestrones	↑ *	↑ **
E/A ratio	↑ ***	↑

Figure 1. Variations of tissue aromatase activity, estrogen/androgen ratio (E/A), and estrogen metabolites (hydroxyestrones) in men and women with RA and SLE. *Urine and synovial fluid, **urine and serum, ***serum and synovial fluid.

See Patients with RA and SLE have increased renal excretion of mitogenic estrogens in relation to endogenous antiestrogens, page 489

patients might indicate that it is also not disease-specific. Further, E2 is thought to play both pro- and antiinflammatory roles in chronic inflammatory diseases that were found to be related to low and high concentrations, respectively.

In light of these data, it is possible that the phenomenon might depend on different dose-related rates of peripheral E2 conversion to pro- or antiinflammatory metabolites, such as 16-hydroxyestrone or naturally occurring antagonists (i.e., 2-hydroxyestrogens), respectively.

Estrogenic status in autoimmune diseases

Further findings suggest an accelerated metabolic conversion of upstream androgen precursors to E2 in RA and SLE patients. E2, as the aromatic endproduct of the gonadal steroid metabolic pathway and the result of peripheral conversion from the adrenal androgen dehydroepiandrosterone (DHEA), recognizes DHEA, testosterone, and progesterone as upstream precursors. Indeed, a large number of studies and reviews in the last 20 years have described reduced serum concentrations of DHEAS, testosterone, and progesterone in both male and female patients with RA and SLE^{12,13}. These data strongly support an accelerated peripheral metabolic conversion of upstream androgen precursors to E2 (Figure 2).

High estrogen concentrations have been found in particular in SF of RA patients. How can one explain the recent detection of lower androgen and higher estrogen levels in SF from both female and male patients with RA? The appropriate explanation might originate from recent studies showing that the inflammatory cytokines — tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6), and IL-1 — are particularly increased in RA synovitis, and are able to markedly stimulate the aromatase activity in peripheral tissues^{14,15}. Indeed, the

aromatase enzyme complex is involved in the peripheral conversion of androgens (testosterone and androstenedione) to estrogens (estrone and estradiol, respectively). In tissues rich in macrophages a significant correlation was found between the aromatase activity and IL-6 production, and aromatase has also been found in synoviocytes¹⁶. Therefore, the increased aromatase activity induced by locally produced inflammatory cytokines (TNF- α , IL-1, IL-6) might explain the altered balance resulting in lower androgens and higher estrogens in RA SF, as well as their effects on synovial cells, as we described⁷ (Figure 1).

The role of local sex hormone concentrations at the level of inflammatory foci is of great value in explaining the modulatory effects exerted by these hormones on the immune-inflammatory reaction. In patients with SLE the aromatase activity evaluated in skin and subcutaneous tissue showed a tendency toward an increase compared to control subjects¹⁷. Among SLE patients, the aromatase activity varied inversely with disease activity, and the patients had decreased androgen and increased serum estrogen levels. Thus tissue aromatase activity showed a significant direct correlation with estrogen levels in patients with SLE.

These data suggest that abnormal regulation of aromatase activity (i.e., increased activity) may partially explain the abnormalities of peripheral estrogen synthesis (i.e., increased availability of E2 and possible metabolites) in SLE, as well as the altered serum sex hormone levels and ratio (i.e., decreased androgens and DHEAS; Figure 1).

Similarly, in the study from Straub's group it is thought that the urinary excretion of hydroxyestrogens (namely, 16-hydroxyestrone and 2-hydroxyestrogens) reflects the production in the tissues, since no respective hydroxylase activity is expected in the urine.

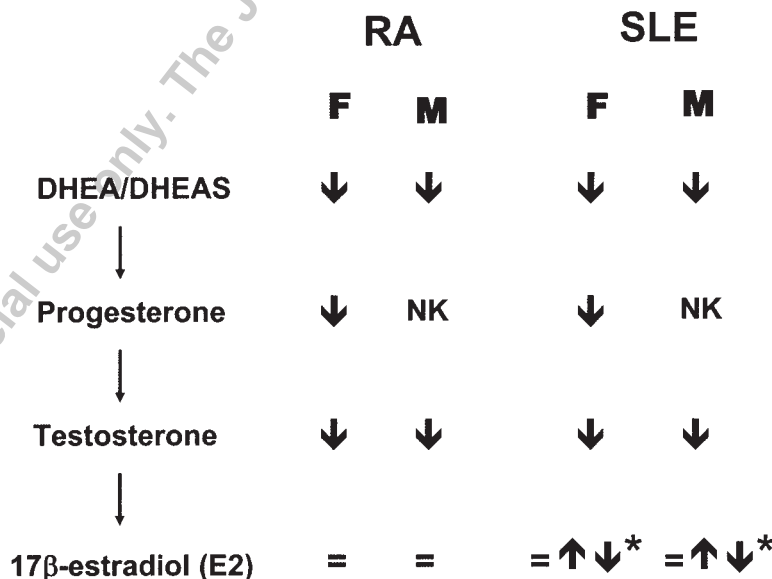


Figure 2. Metabolic pathways in steroid conversion (left). Variations of body fluid concentrations of DHEA, progesterone, testosterone, and 17 β -estradiol (E2) in men and women with RA and SLE. *Changes observed within physiological ranges. NK: not known.

On the other hand, as recently reviewed, peripheral estrogen hydroxylation was found to be increased in both men and women with SLE, and estrogenic metabolites were reported to increase B cell differentiation and activate T cells¹⁸.

Testing possible mechanisms

Recent studies have shown that 16-hydroxyestrone was far more potent than E2 in exerting proliferative activities³. Recently in cultured human myeloid monocytic cells (THP-1) differentiated into activated macrophages, we tested the effects of E2 and testosterone to evaluate their influence on cell proliferation and apoptosis. The effects were evaluated on nuclear factor- κ B (NF- κ B) pathway, as a complex of molecules modulating cellular activation. Testosterone was found to exert pro-apoptotic effects and to reduce activated macrophage proliferation, whereas E2 induced the opposite effects: both hormones interfered with the activities of NF- κ B pathway (submitted for publication). These results might support the hypothesis of a sex hormone modulation of cell growth and apoptosis. The next step will be to test the effects of 16-hydroxyestrone and 2-hydroxyestrogens on the same cells.

In another study, E2 was found to increase IgG and IgM production by peripheral blood mononuclear cells (PBMC) from patients with SLE, which led to elevated levels of polyclonal IgG, including IgG anti-double strand-DNA, by enhancing B cell activity via IL-10¹⁹. These latter results should also be replicated in the presence of 16-hydroxyestrone, as well as with the naturally occurring 2-hydroxylated antiestrogen. Indeed, Straub's group showed that disease activity in SLE was negatively correlated to urinary concentrations of 2-hydroxylated estrogens¹.

Thus, sex hormones can exert local actions (paracrine) in the tissues in which they are formed, and an accelerated peripheral metabolic conversion of upstream androgen precursors to E2 and to even more estrogenic metabolites can be observed, at least in patients with RA and SLE. Estrogens are confirmed as one of the risk factors in autoimmunity. All these data suggest caution in exogenous estrogen administration in patients with autoimmune diseases (i.e., oral contraceptives, estrogen replacements, induction of ovulation), yet raise the prospect of novel and improved applications of hormonal or antihormonal immunotherapy, such as antiestrogens, receptor modulators, antagonist metabolites, and androgenic compounds.

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