Patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus Have Increased Renal Excretion of Mitogenic Estrogens in Relation to Endogenous Antiestrogens

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ABSTRACT. ***Objective***. In patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), 17ß-estradiol was thought to play a dual pro- and antiinflammatory role depending on its concentration or probably conversion to downstream mitogenic 16α-hydroxyestrone or naturally occurring antiestrogens such as 2-hydroxyestrone. We compared renal excretion of these 2 types of estrogens in healthy subjects and patients with RA and SLE.

Methods. In a prospective study with 30 patients with RA, 32 with SLE, and 54 healthy subjects, we measured urinary levels of 16α-hydroxyestrone and 2-hydroxyestrogens by enzyme immunoassay. We studied renal excretion to estimate the time-integral of hormone production.

Results. Urinary concentration and total urinary loss of 2-hydroxyestrogens was 10 times higher in healthy subjects compared to patients with either SLE or RA irrespective of prior prednisolone treatment or sex. The urinary concentration and loss of 16α-hydroxyestrone did not differ between healthy subjects and patients with RA/SLE. The ratio of urinary 16α-hydroxyestrone/2-hydroxyestrogens was more than 20 times higher in RA and SLE than healthy subjects irrespective of prior glucocorticoid treatment or sex.

Conclusion. This study in RA and SLE patients clearly demonstrates a large shift to mitogenic estrogens in relation to endogenous antiestrogens. Both steroids are converted from the precursor 17ß-estradiol and estrone. In patients with RA and SLE, the magnitude of conversion to the mitogenic 16α-hydroxyestrone is greatly upregulated, which likely contributes to maintenance of the proliferative state in these diseases. (J Rheumatol 2004;31:489–94)

Key Indexing Terms:
RHEUMATOID ARTHRITIS ESTROGENS SYSTEMIC LUPUS ERYTHEMATOSUS
16α-HYDROXYESTRONE 2-HYDROXYESTRONE 2-HYDROXYESTRADIOL

In the late 1970s, Kunkel and colleagues made the important observation that estrogen metabolism is altered in patients with systemic lupus erythematosus (SLE)[1,2]. This group described elevated serum concentrations of 16α-hydroxylated estrogens in patients with SLE. They concluded that women with SLE had abnormal patterns of estradiol metabolism that may lead to increased estrogenic activity[1]. A similar phenomenon was recently described for synovial fluid concentrations of this hormone in patients with rheumatoid arthritis (RA)[3]. This suggests that 16α-hydroxylated estrone may play an important role in these diseases. This estrogen and others are converted from upstream estrone and 17ß-estradiol (Figure 1).

Interestingly, breast cancer research revealed a mitogenic tumor growth-stimulating role of 16α-hydroxylated estrogens that indicates the potent estrogenic activity of these hormones[4]. These hormones bind to the estrogen receptor and thereby induce nuclear translocation of the hormone-receptor complex and subsequently growth of breast cancer cells *in vitro*[5]. Other conversion products of estrone and 17ß-estradiol are the 2-hydroxylated estrogens such as 2-hydroxyestrone and 2-hydroxyestradiol (Figure 1). In contrast to 16α-hydroxylated estrogens, the 2-hydroxylated forms inhibit growth-promoting effects of 17ß-estradiol[6]. The anticarcinogenic effect of 2-hydroxyestrone has been extensively reviewed[7]. Further, the estrogen metabolism pathway favoring 2-hydroxylation over 16α-hydroxylation is associated with a reduced risk of invasive breast cancer in

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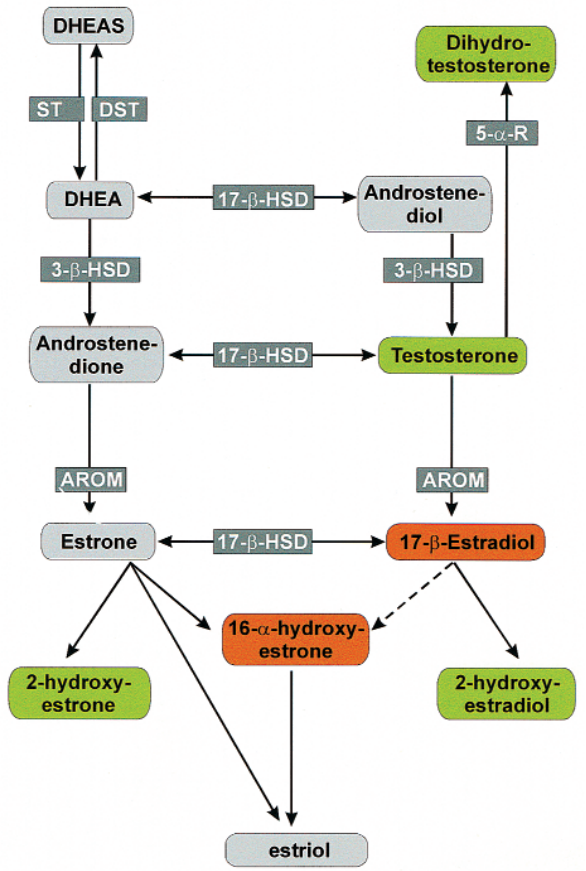

Figure 1. The biosynthesis of steroid hormones. Precursor hormones such as DHEAS and DHEA can be converted to downstream androgens (androstenedione, testosterone) and estrogens [hormones below the aromatase enzyme (AROM)]. Steroid hormones shown in green (red) indicate supposed antiinflammatory (proinflammatory) activity. DHEA: dehydroepiandrosterone; DHEAS: DHEA sulfate. Enzymes: 3-ß-HSD: 3ß-hydroxysteroid dehydrogenase; 5-α-R: 5α-reductase; 17-ß-HSD: 17ß-hydroxysteroid dehydrogenase; DST: DHEA sulfatase; ST: sulfotransferase.

premenopausal women[8]. In epithelial cells, 16α-hydroxyestrone leads to transformation and increased proliferative activity[9].

We can summarize that 16α-hydroxylated estrogens are biologically active whereas the 2-hydroxylated metabolites of estrone and 17ß-estradiol are not. Thus, the molar relationship of 2-hydroxylated versus 16α-hydroxylated estrogens may be an important indicator of the presence of inactive (or even antagonizing) versus active estrogens. In patients with RA and SLE, we investigated the renal excretion of both metabolites and their molar ratio. Renal excretion yields a time-integral of the endogenous production of these estrogenic metabolites and is more suitable compared to single measurements in serum.

MATERIALS AND METHODS

We studied 30 Caucasian patients with diagnosed RA fulfilling the American College of Rheumatology criteria[10]. Clinical variables of disease activity included the number of swollen and tender joints and erythrocyte sedimentation rate. In order to simultaneously study patients with another chronic inflammatory disease, we enrolled 32 Caucasian patients with SLE according to the criteria of the American College of Rheumatology[11]. In the latter, clinical activity was assessed by the SLE Disease Activity Index (SLEDAI). Basic characteristics of both disease groups, including therapy, are shown in Table 1. It can be seen that patients in both disease groups presented mild to moderate disease activity. All women with periodic menstrual bleeding were in the follicular phase of the menstrual cycle.

For comparison, 54 healthy Caucasian controls were recruited (mean age 40.8 ± 1.5 yrs), and health status was verified by means of a 33-item questionnaire[12]. The questionnaire addressed known diseases in the past and at present, current symptoms of disease, current medication, alcohol intake, smoking habits, family history, and surgical history. The questionnaire was adapted to the SENIEUR protocol[13]. Our rules established strict admission criteria for immunogerontological studies based on clinical information. Fertile women (controls and patients) were not taking contraceptives and they were in the early to mid-follicular phase of the menstrual cycle.

Due to the different ages and sexes in the disease groups, subgroup analyses were carried out in order to correctly compare the different groups with controls. Subgroups were matched according to age and sex (Table 1).

Patients and controls were instructed to collect urine for 24 h in special urine containers during the day before the visit. On the day of the visit, between 8:00 and 10:00 AM, urine samples for further determination of steroid hormones were taken and stored at –30°C in adequate aliquots. All patients and controls gave written consent for further investigation of samples. Due to the difficulties in recruiting large numbers of patients and controls (taking no contraceptives and in the correct phase of the menstrual cycle), collection of the material lasted 2 years.

Laboratory measures. We used the Estramet 2/16 commercial enzyme immunoassay kit (IBL, Hamburg, Germany). This assay detects either 16α-hydroxyestrone (cross-reactivity of the subject antibody for other related estrogens and androgens at least < 3%) or the 2-hydroxylated forms of estrone (cross-reactivity of the antibody 100%), estradiol (100%), and estriol (74%). Since urinary forms of these hormones appear as glucuronides or sulfates, the estrogens were deconjugated by use of a mixture of ß-glucuronidase and arylsulfatase (Gusulase; IBL). The assay and antibodies are described by Klug, *et al*[14]. The sensitivity of the assay is about 0.2 nmol/l urinary 16α-hydroxyestrone and roughly 0.5 nmol/l of 2-hydroxyestrogens. For both assays, intraassay and interassay coefficients of variation were below 10%. Both assays have been shown to demonstrate 100% recovery of metabolites. Urinary levels of these 2 hormones are good indicators of their production. Since serum concentrations cannot be measured by this test kit, we were unable to determine serum levels of these 2 hormones.

Patients and controls were also part of another related study that investigated excretion of adrenal hormones (unpublished data). That study revealed that creatinine clearances of controls and patients in the different disease groups were very similar. Thus, we do not give these data here.

Statistical analysis. To compare medians in 2 different groups the Mann-Whitney signed-rank test was used (SPSS/PC for Windows, V. 10.0.5, SPSS Inc., Chicago, IL, USA). Investigation of interrelation between 2 variables was by Spearman rank correlation analysis. P values less than 0.05 were considered significant and means are given ± SEM.

RESULTS

Urinary hormone concentration of 2- and 16α-hydroxylated estrogens. In comparison to controls, patients with RA and SLE irrespective of prior prednisolone treatment had significantly lower urinary hormone concentrations of 2-hydroxyestrogens (Figures 2A, 2B). The levels in controls were 10 to 20 times higher compared to patients with RA and SLE. In contrast, we found no marked difference in urinary levels

Table 1. Basic characteristics of patients with RA or SLE in the subgroup analyses. Data are given as means ± SEM and percentages in parentheses.

	RA	SLE	Controls
Number	30		23
Age, yrs	56.1 ± 2.4		51.5 ± 0.8
Sex (f/m), n	22/8 (73/27)		12/11 (52/48)
Premenopausal women, n	3/22		1/12
Disease duration, yrs	10.8 ± 1.9		NA
No. of swollen joints	4.1 ± 1.5		NA
No. of painful joints	6.6 ± 1.6		NA
ESR, mm/h	27.7 ± 3.9		NM
Medication			
Prednisolone	20 (67)		NA
Prednisolone, mg/day	6.5 ± 1.8		NA
Methotrexate	9 (41)		NA
NSAID	11 (38)		NA
Leflunomide	8 (27)		NA
Anti-TNF strategy	6 (21)		NA
Sulfasalazine	2 (7)		NA
Cyclophosphamide	0 (0)		NA
Azathioprine	0 (0)		NA
Number		32	42
Age, yrs		38.1 ± 2.1	37.1 ± 1.5
Sex (f/m), n		24/8 (75/25)	25/17 (60/40)
Premenopausal women		22/24	18/25
Disease duration, yrs		8.0 ± 1.5	NA
SLEDAI (points)		10.1 ± 1.5	NA
ESR, mm/h		25.0 ± 3.3	NM
Medication			
Prednisolone		22 (69)	NA
Prednisolone, mg/day		9.4 ± 3.4	NA
Methotrexate		2 (6)	NA
NSAID		13 (41)	NA
Leflunomide		0 (0)	NA
Anti-TNF strategy		NA	NA
Sulfasalazine		0 (0)	NA
Cyclophosphamide		2 (6)	NA
Azathioprine		12 (38)	NA

Anti-TNF strategies are either infliximab or etanercept. ESR: erythrocyte sedimentation rate; NA: not applicable; NM: not measured; NSAID: nonsteroidal antiinflammatory drugs; SLEDAI: SLE Disease Activity Index.

of 16α-hydroxylated estrone between controls and patients of both disease groups (Figures 2C, 2D). Comparison of women and men with RA/SLE with their respective controls revealed very similar results (data not shown). Comparison of male versus female controls and also patients did not reveal a significant difference for urinary hormone concentrations of the 2 metabolites (data not shown).

Total urinary loss of 2- and 16α-hydroxylated estrogens. A very similar picture to that for urinary concentrations was found for total urinary loss of these hormones in nmol per hour (Figure 3). Urine volume and collection time were included into the calculation of this measure, as: total loss = (concentration × volume)/collection time. Again, patients in both disease groups irrespective of prior prednisolone treatment compared to controls showed a decreased loss of 2-hydroxylated estrogens (Figures 3A, 3B). In patients with RA, the molar amount of excreted 16α-hydroxyestrone was very similar to controls (Figure 3C). In SLE patients without prior prednisolone treatment, loss of 16α-hydroxyestrone was significantly increased in relation to controls (Figure 3D). This did not reach significance level in all SLE patients including those with prednisolone pretreatment due to the high variation (Figure 3D). Comparison of women and men with RA/SLE to their respective controls revealed very similar results (data not shown). Comparison of male with female controls and also patients revealed no significant difference for both urinary hormone concentrations (data not shown).

Relation of 16α-hydroxylated and 2-hydroxylated estrogens. To estimate the relative increase of 16α-hydroxyestrone in relation to 2-hydroxylated estrogens a molar ratio of these 2 hormones was calculated using the numerical value of the urinary concentrations (an identical ratio is calculated when the total urinary loss in nmol/h is used; i.e., a unitless ratio).

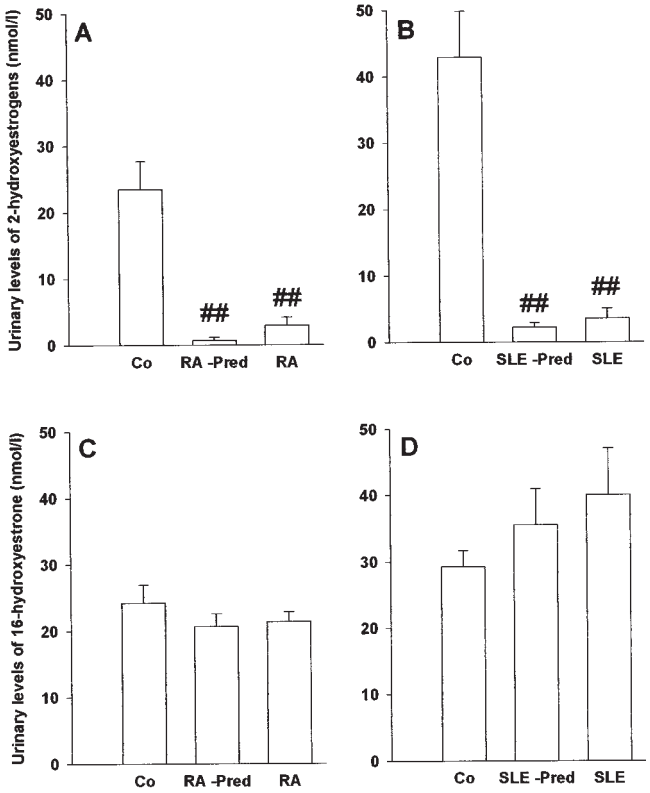

Figure 2. Urinary concentrations of 2-hydroxylated estrogens (A, B) and 16α-hydroxyestrone (C, D) in patients with RA and SLE. Co: healthy controls; –Pred: no prior prednisolone therapy. $^{\#\#}p < 0.001$ for comparison of the respective median with control. Data are given as mean nmol/l ± SEM.

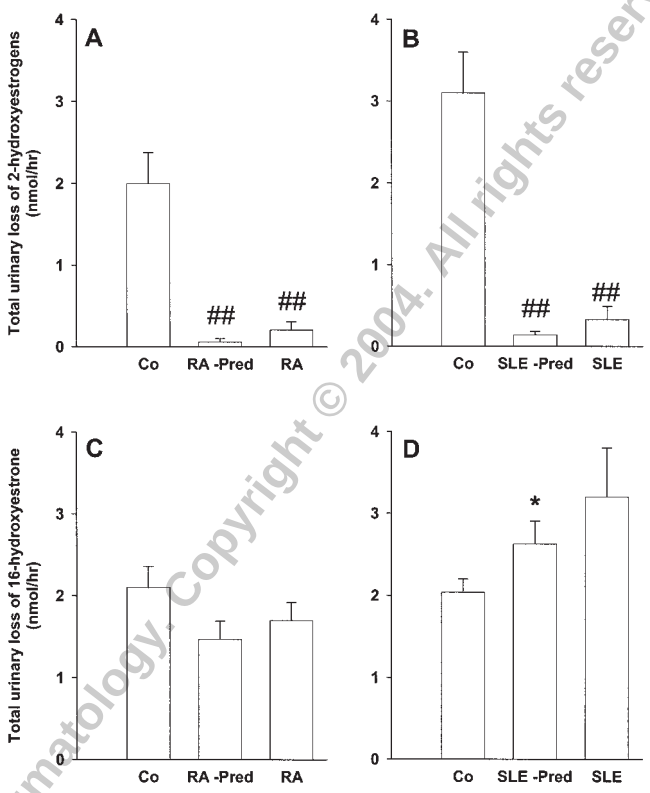

Figure 3. Total urinary loss of 2-hydroxylated estrogens (A, B) and 16α-hydroxyestrone (C, D) in patients with RA and SLE. Co: healthy controls; –Pred: no prior prednisolone therapy. $^{*}p < 0.05$, $^{\#\#}p < 0.001$ for comparison of the respective median with control. Data are given as mean nmol/h ± SEM.

This ratio was elevated more than 20 times in both patient groups without prior prednisolone treatment, and it was even further enhanced in all patients of the 2 disease groups (Figure 4). Thus, the molar amount of excreted 16α-hydroxyestrone is markedly elevated in relation to 2-hydroxylated estrogens. Comparison of women and men with RA/SLE with their respective controls yielded the same results (data not shown). Similar to the urinary hormone concentrations and total renal excretion, this phenomenon was not sex-dependent.

Disease activity and excreted 2- and 16α-hydroxylated estrogens. In patients with RA, irrespective of prior prednisolone treatment, excretion of both estrogen metabolites was not related to the number of tender or swollen joints ($p \geq 0.10$). In all patients with SLE, the total molar amount of 2-hydroxylated estrogens per hour was negatively correlated with the SLEDAI ($R_{Rank} = -0.389$, $p = 0.045$), but no interrelation was observed for 16α-hydroxyestrone ($p > 0.10$). In the smaller number of SLE patients without prior prednisolone treatment, this did not achieve significance ($p > 0.10$).

DISCUSSION

This study clearly demonstrates increased renal excretion of 16α-hydroxyestrone in relation to 2-hydroxylated estrogens, which may be unfavorable. Considering recent studies[8,15], it is thought that urinary excretion of these hormones reflects production in the tissue because no respective hydroxylase activity is expected in the urine. We recently reported that patients with RA present significantly elevated concentrations of synovial 16α-hydroxyestrone and 4-hydroxyestradiol compared to controls[3]. The synovial fluid levels of 2-hydroxyestrone were not significantly different between the 2 groups. Thus, local conversion of estrone and 17ß-estradiol are most likely the source of 16α-hydroxyestrone and 2-hydroxylated estrogens. The conversion may be particularly upregulated in inflamed tissue[3].

Studies in breast cancer research determined that 16α-hydroxyestrone is a mitogenic and proliferative endogenous hormone that binds covalently to the estrogen receptor, leading to nuclear translocation[4,5,9]. Because of this covalent linkage to the receptor, 16α-hydroxyestrone shows persistent biological responses[16]. In proliferation assays, 16α-

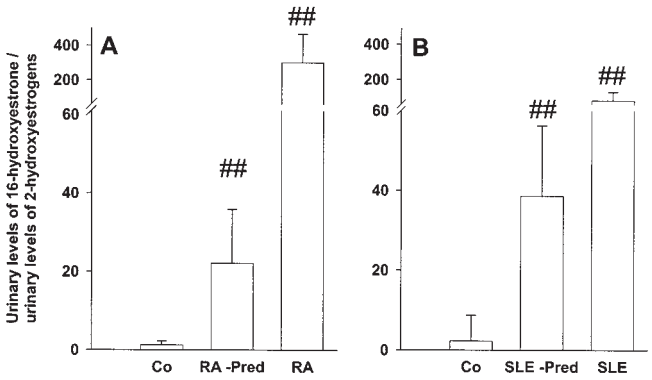

Figure 4. Molar ratio of 16α-hydroxyestrone and 2-hydroxylated estrogens in patients with RA (panel A) and SLE (panel B). Co: healthy controls; –Pred: no prior prednisolone therapy. ##p < 0.001 for comparison of the respective median with control. Data are given as mean ± SEM (without unit).

hydroxyestrone had an activity comparable to that observed for the carcinogen DMBA[9]. Measurement of anchorage-independent colony formation of mammary epithelial cells grown in soft agar showed that 16α-hydroxyestrone was far more potent than 17ß-estradiol[9]. Thus, 16α-hydroxyestrone may induce a hyperestrogenic state. This is particularly true if the naturally occurring antiestrogens, the 2-hydroxylated estrogens, are diminished. This is obviously the case in both patient groups in our study, which led to more than 20 times increased molar amounts of 16α-hydroxyestrone in relation to 2-hydroxylated estrogens. In contrast to 16α-hydroxylated estrogens, the 2-hydroxylated forms inhibit growth-promoting effects of 17ß-estradiol[6]. In this respect, 2-hydroxyestrone has anticarcinogenic properties and, thus it is most likely a naturally-occurring antiestrogen[7]. The relative loss of 2-hydroxylated estrogens in relation to 16α-hydroxyestrone may thus be an important switch to support the chronic proliferative state in these diseases. This is corroborated by the observation that disease activity in patients with SLE was negatively correlated to urinary concentrations of 2-hydroxylated estrogens. One may speculate that the role of 17ß-estradiol, which was thought to play a dual pro- and antiinflammatory role in chronic inflammatory diseases depending on its local concentration[17,18], largely depends on conversion downstream to pro- or antiinflammatory metabolites such as 16α-hydroxyestrone or naturally-occurring antagonists (2-hydroxylated forms of estrogens). Further, conversion to metabolites downstream may also determine the pro- and antiinflammatory role of estrogens during hormone replacement therapy. Since the excretion of 2-hydroxylated estrogens was very low in the 2 disease groups, we may expect an important shunt pathway for estrogens, and elucidation of this would be important to understand the entire phenomenon.

It is intriguing that sex and the ovulatory state did not influence our findings. This clearly indicates that the gonadal production of these hormones is not responsible for the observed results. Thus, it seems that most of the measured metabolites are converted in the periphery, which is largely independent of sex. It is most probable that this mainly depends on the inflammatory state in the tissue[3]. Moreover, the hormone shift appears in both disease groups, which indicates that the phenomenon is not disease-specific. Similarly disease-nonspecific is the well known loss of adrenal androgens and the inadequately low serum levels of cortisol in relation to inflammation. It seems that these disease related changes are not evolutionarily conserved for a specific inflammatory disease. Most probably these inflammation-dependent reactions were evolutionarily conserved for other processes, which are used in a large number of inflammatory reactions. Discovery of the general rule behind these phenomena must be of great future interest.

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