TESTOSTERONE METABOLISM AND CYCLOSPORIN A TREATMENT IN RHEUMATOID ARTHRITIS

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SUMMARY

A constant dose-dependent side-effect in cyclosporin A (CSA)-treated patients is the appearance of hypertrichosis; this occurs in both sexes and suggests an androgenizing activity. To determine the influence of CSA on peripheral androgen metabolism, we evaluated in rheumatoid arthritis (RA) patients treated with low-dose CSA (3.5 mg/kg/day), during a period of 12 months, plasma levels of testosterone (Tes) and of 5α-androstane-3α, 17β-diol glucuronide (Adiol-G), an important peripheral Tes metabolite. Clinical and laboratory parameters of RA were also monitored. Furthermore, the metabolism of physiological concentrations of Tes (1 × 10⁻⁸ M) was evaluated in primary cultures of RA synovial macrophages (Mₘ) in the presence of CSA concentrations close to the pharmacological immunosuppressive doses (100–500 ng/ml). At the final time of observation (12 months), a significant increase in the mean plasma Adiol-G level was observed in patients of both sexes. The increase was evident after 1 month of treatment in male patients (P < 0.01) and after 3 months in female patients (P < 0.05). Almost all the patients experienced the side-effect of a low-degree hypertrichosis after a mean period of 1–2 months. No significant correlations with the laboratory parameters of the disease were observed. Results from in vitro experiments on Tes metabolism by cultured synovial Mₘ showed at 24 and 48 h, in the presence of CSA, a significantly (P < 0.0001) greater formation of dihydrotestosterone and increased amounts of other Tes metabolites, including androstenedione, androsterone and epiandrosterone, when compared to untreated controls. In conclusion, the appearance of a dose-related hypertrichosis and the increase in plasma androgen metabolites (i.e. Adiol-G) in CSA-treated patients, as well as the hormonal metabolic effects on cultured synovial Mₘ, should be regarded as possible markers of the influence of CSA on peripheral androgen metabolism at the level of target cells.

KEY WORDS: Testosterone, Androgens, Rheumatoid arthritis, Synoviocytes, Macrophages, Cyclosporin A, Hypertrichosis.

Cyclosporin A (CSA) is the first non-steroidal drug having clearly specific immunosuppressive activities that has been used extensively in the prevention of allograft rejection, as well as in the treatment of immunomodulated diseases, including rheumatoid arthritis (RA), psoriasis (Ps) and psoriatic arthritis (PsA) [1–4]. CSA may have more than one mode of action and can act on different cell types involved in the immune response, including T lymphocytes and antigen-presenting cells (APCs) such as macrophages [5, 6]. A nearly constant dose-dependent side-effect in CSA-treated patients is the appearance of hypertrichosis, suggesting an androgenizing activity [7]. However, previous studies failed to demonstrate increased plasma androgens in patients treated with CSA [8]. On the contrary, decreased plasma testosterone (Tes) in long-term treatment has been described [9]. Recent reports have suggested a dose-related influence of CSA administration on Tes peripheral metabolism in patients affected by insulin-dependent diabetes (IDD) and PsA [9, 10]. Tes, and its derived metabolite dihydrotestosterone (DHT), may exert suppressive activities on androgen receptor-positive cells involved in the immune response, including macrophages [11, 12]. In particular, it is suggested that androgens might exert immunosuppressive activities in human and murine synovial macrophages (Mₘ) that are androgen receptor-positive cells able to metabolize Tes and to influence cytokine production [13–19].

Based on these observations, we decided to investigate the plasma concentrations of Tes and 5α-androstane-3α, 17β-diol glucuronide (Adiol-G), an important Tes metabolite, in patients affected by RA and treated with low-dose CSA (3.5 mg/kg/day), during a period of 12 months. Adiol-G is considered a specific hormonal marker in ‘idiopathic hypertrichosis’ and the increase in plasma Adiol-G was suggested to be associated with the hypertrichosis in CSA-treated patients [9, 10]. To verify in vitro the influence exerted by CSA on androgen metabolism, we evaluated metabolic pathways of Tes in primary cultures of RA synovial Mₘ in the presence of pharmacological concentrations of CSA.

MATERIALS AND METHODS

Patients

Eleven patients (six men and five women, mean age ± s.d. = 48 ± 5 yr) fulfilling the American College of Rheumatology criteria for adult RA were entered into the study after informed consent was obtained [20]. All patients were unresponsive or very poorly
responsible to conventional treatment, and the arthritis was not adequately controlled by non-steroidal anti-inflammatory drugs. Patients were excluded from the study with CSA in the presence of one or more of the following conditions, in agreement with recent International Consensus Conference indications: previous treatment with CSA, sulphasalazine, methotrexate, etretinate, or other immunosuppressive agents within at least 8 weeks before proposed entry into the study, serum creatinine > 110 mmol/l, proteinuria > 0.5 g/24 h, leucopenia, thrombocytopenia or abnormal liver function test, hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 100 mmHg after 5 min in a sitting position), malignancy or history of malignancy, immunodeficiency, uncontrolled infections, pregnancy, breast feeding, impairment of cardiovascular or cerebral function, IDD, drug or alcohol abuse [21]. None of the patients received other drugs reported to induce hypertrichosis or hirsutism [22].

Cyclosporin A treatment
CSA, divided into two oral doses, was generally started at 3.5 mg/kg/day. The dose of CSA had to be reduced by 0.5 mg/kg/day if there was an increase in serum creatinine of > 30% above basal values, hypertension, not tolerated hypertrichosis or gingival hyperplasia. The CSA dose was reduced by 25% if total bilirubin or liver transaminases increased by > 100% over baseline. Patients were allowed to continue their non-steroidal anti-inflammatory therapy during the study.

Assessment of disease activity
Each patient was examined monthly by the same rheumatologist for the first 3 months, thereafter every 3 months for 12 months. At each visit, the number of swollen joints and the number of tender joints were evaluated. Patients were monitored for the appearance of hypertension and carefully examined for gingival hyperplasia. Hypertrichosis was evaluated following the Ferriman and Gallwey score [23]. Laboratory parameters to assess CSA therapeutic activity and tolerance included complete blood and differential count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum creatinine, liver transaminases and alkaline phosphatase. The analyses were performed by standard methods at baseline and every 4–8 weeks.

Plasma androgen assays
After collection (at baseline, every week for the first month and every 4 weeks thereafter) plasma for hormonal analysis was frozen at −10°C until assay. Plasma Adiol-G was measured by radioimmunoassay (by evaluating both 3α and 17β androstanediol glucuronide) without previous extraction (Diagnostic System Laboratories Inc., Webster, TX, USA). Mean concentrations of Adiol-G established in normal controls were 3.32 ± 1.80 nmol/l in pre-menopausal women (range 1.06–11.50 nmol/l), 2.19 ± 1.19 nmol/l in post-menopausal women (range 0.21–12.80 nmol/l) and 6.04 ± 3 nmol/l in men (range 4.24–38.86 nmol/l). The lowest detectable level of Adiol-G was 0.25 nmol/l at the 95% confidence limit. An inter-assay coefficient of variation of 7.3% and an intra-assay coefficient of variation of 6.7% were found. Serum total Tes was measured by direct immunoassay as previously described [24]. Mean concentrations of total Tes established in normal women and men were 0.78 ± 0.27 and 20.10 ± 4.50 s.e. nmol/l, respectively. An inter-assay coefficient of variation of 7% was found.

Preparation of synovial macrophage culture
Synovial tissue samples were obtained from three women (mean age 49.6 ± 14 yr) and three men (mean age 53.7 ± 15 yr) who fulfilled the American College of Rheumatology criteria for adult RA and were undergoing surgical synovectomy of the knee [20, 25]. All the RA patients were in Steinbrocker class III.

At the time of surgery, all of the patients were being treated exclusively with non-steroidal anti-inflammatory drugs. None had received any oral or intra-articular corticosteroid therapy or disease-modifying anti-rheumatic drugs during the 4 months preceding the investigation, nor was the use of the oral contraceptive pill, 1,25-dihydroxyvitamin D3 or any hormone replacement therapy allowed [26–28]. All subjects had normal liver, renal, prostate and thyroid functions, and were within 20% of ideal body weight.

The synovial tissue, soon after surgery, was dissected away from fatty, capsular and cartilaginous components, cut into 3–5 mm pieces, washed in Hank’s balanced salt solution and incubated in collagenase (1 mg/ml; Sigma Chemical Co., St Louis, MO, USA) for 3 h at 37°C with constant agitation. The digest was then passed through a wire mesh with a pore size of 200 μm to separate dissociated cells from tissue debris. The cells were washed twice in collagenase and counted. The mean cell yield was 4.9 × 10⁶ (range 0.53–16.8 × 10³) and the mean viability was 88% (range 69–98%). The cells were then cultured with RPMI supplemented with 10% fetal calf serum (FCS) at 37°C in a humidified 5% CO₂ atmosphere in air. After 3 h, non-adherent cells were removed and the adherent cells were cultured with RPMI supplemented with 10% FCS at 37°C in a humidified 5% CO₂ atmosphere in air for 24 h. Every 24 h, cytocentrifuge preparations (800 g × 3 min) of suspended cells (gentle scraping) were obtained and stained as previously reported with specific monoclonal antibodies directed toward the macrophage-specific antigens (Ber-MAC3; Dakopatts, Copenhagen, Denmark); they were found to be > 90% Mφ [14].

Testosterone metabolism in primary cultures of synovial macrophages
Methodological approaches and procedures used to measure metabolic pathways of steroids have been established and optimized previously [29]. Adherent synoviocytes were harvested by mild
trial, trypsinization, counted in a haemocytometer and plated onto 6-well tissue culture plates at a density of 0.5–1 × 10^6 cells/dish. After 24 h, cells were washed twice with PBS-A and the medium substituted with 2 mL FCS-free, phenol red-free RPMI medium, containing 10^-4 M tritiated Tes as precursor (1,2,6,7-^3H(N)T; sp. act. 92.1 Ci/mmol; DuPont de Nemours Italiana Spa, Milan, Italy). The tritiated precursor was periodically checked using high-performance liquid chromatography (HPLC) prior to experimental use. Furthermore, parallel dishes with medium plus precursor without cells were used to control precursor degradation during each experiment. Parallel dishes received two different concentrations of CSA (100 and 500 ng/ml) (Sandoz Ltd, Basel, Switzerland) throughout the culture period. Following 24–48 h incubation, the medium was transferred to sterile plastic tubes (Costar) and stored at −80°C until analysis. Aliquots (100 μl) of the cell lysates were therefore used to estimate DNA content, as described elsewhere [30]. Androgen extraction was carried out on 1 ml of medium with 10 ml of diethyl ether. After mixing at 3°C for 30 min, the resulting aqueous phase was freeze-dried in a Speed Vac evaporator (Savant Instruments Inc., Farmingdale, NY, USA) and then resuspended using 970 μl of acetate buffer (0.75 M, pH 5.0) added with 30 μl of gluclase enzyme mixture (DuPont Co., Wilmington, DE, USA) and incubated at 37°C for 18 h to hydrolyse androgen conjugates (sulphates and glucuronides). Following incubation, samples were transferred to glass vials and extracted again using 10 ml diethyl ether as before. The two ether phases (free and hydrolysed steroids) were evaporated to dryness under nitrogen and then stored at −20°C until required for analysis using reverse-phase high-performance liquid chromatography (RP-HPLC). The HPLC system consisted of a Model 324 system, equipped with a Model 160 UV detector set at 280 nm (Beckman Instruments Inc., Berkeley, CA, USA) and with an ‘on-line’ Flo-One/ beta (Model 1C) three-channel radiometric detector (Radiomatic Instruments, High Wycombe). Steroids were separated under isocratic conditions using a Spherisorb ODS-2 (Aldrich Chimica, Milan, Italy) column (250 × 4.6 mm ID) and 45% acetonitrile in citric acid (0.05 M) as optimized mobile phases for androgen separation, with a flow rate of 1 ml/min. Routine data integration was automatically achieved and computed in net c.p.m. by a Flo-One/beta F0B program (Radiomatic, Tampa, FL, USA). Limits for radiometric detection were ~50 c.p.m. (corresponding to 1.1 fmol) using [1,2,6,7-^3H]Tes (sp. act. 92.1) as also reported previously [29]. The Tes metabolites evaluated included DHT, androstenedione (A4-Ad), androstane-dione (5d-Ad), 5α-androstane-3z,17β-diol (Adiol), androsterone (A) and epiandrosterone (Epi-A).

**Statistical analysis**

Non-parametric procedures were employed to evaluate the statistical significance between basal and final observation times for the considered values. Statistical analysis was carried out using the time series analysis and the correlation coefficient between the hormonal parameters. Results on plasma steroid concentrations were assessed by paired t-test in individuals. The two-tailed paired Student’s t-test (95% confidence limits) was used to compare Tes metabolic rates in controls and CSA-treated synovial Mφ.

**RESULTS**

**Plasma androgen variations and clinical response**

In male RA patients, a significant increase in the mean plasma Adiol-G concentrations (P < 0.01) was observed after 1 month of CSA treatment (see Table I). The increase was confirmed after 3 and 12 months of treatment (P < 0.05 and P < 0.001, respectively).

In female RA patients, an appreciable increase in the mean plasma Adiol-G concentration was observed after 1 and 2 months of CSA administration, with a significant increase after 3, 6 and 12 months (P < 0.05 and P < 0.001 at 6 and 12 months, respectively) (Table II).

No significant variations of Tes concentrations were

**TABLE I**

Variations of plasma levels of testosterone (Tes) and Adiol-G in six male RA patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiol-G ± s.e. (nmol/l)</td>
<td>4.93 ± 0.83</td>
<td>8.78 ± 1.77*</td>
<td>7.56 ± 1.99</td>
<td>8.90 ± 2.05**</td>
<td>7.93 ± 1.21</td>
<td>9.17 ± 0.72***</td>
</tr>
<tr>
<td>Tes ± s.e. (nmol/l)</td>
<td>19.10 ± 4.67</td>
<td>21.77 ± 5.20</td>
<td>17.95 ± 2.80</td>
<td>20.17 ± 5.23</td>
<td>22.98 ± 6.76</td>
<td>24.47 ± 6.48</td>
</tr>
</tbody>
</table>

*P < 0.01; **P < 0.05; ***P < 0.001.

**TABLE II**

Variations of plasma levels of testosterone (Tes) and Adiol-G in five female RA patients

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiol-G ± s.e. (ng/ml)</td>
<td>2.97 ± 0.63</td>
<td>3.50 ± 0.73</td>
<td>4.14 ± 0.71</td>
<td>4.80 ± 0.83**</td>
<td>6.15 ± 1.97***</td>
<td>5.56 ± 1.58***</td>
</tr>
<tr>
<td>Tes ± s.e. (ng/dl)</td>
<td>0.20 ± 0.06</td>
<td>0.22 ± 0.05</td>
<td>0.20 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.17 ± 0.03</td>
<td>0.18 ± 0.04</td>
</tr>
</tbody>
</table>

**P < 0.05; ***P < 0.001.**
observed during the study in all the patients. Regarding male RA patients, a slight increase in Tes (not significant) was present after 12 months (24.47 vs basal 19.10 nmol/l). Almost all the patients experienced a different degree of hypertrichosis after a mean period of 6–8 weeks. The Ferriman and Gallwey score was lower than eight in all patients before treatment. In particular, in women the mean score changed from 0.9 to 14 above control, respectively, on the other hand, the conversion of Tes into Δ4-Ad was only slightly enhanced by CSA addition (nearly 7% above controls at the 100 ng/ml dose) (see Table III).

Following 48 h incubation, the increase in DHT production was less pronounced, although still significant, at 500 ng/ml (13%) of CSA. In contrast, the Tes conversion to Δ4-Ad was much increased, reaching a peak stimulation of 22% above control with the 100 ng/ml dose (see Table IV). CSA addition produced an opposite effect on the 5α-Ad formation, the latter being inhibited and stimulated at 100 or 500 ng/ml, respectively; however, these differences from controls at both 24 and 48 h were not significant (see Table IV).

The CSA-induced stimulation of DHT production by treated synoviocytes was also corroborated by the formation of Adiol (DHT metabolite) in some assays, although inconsistent and in small amounts. The fact that this DHT derivative is only occasionally observed in vitro may be ascribed to the experimental conditions used (i.e. small cell number, physiological concentrations of labelled precursor, characteristics of conditioned media), probably leading to Adiol levels below the limits of detection.

**DISCUSSION**

In the present study, we have shown that the treatment of RA patients with low-dose CSA induces within a few weeks a significant increase in the mean plasma Adiol-G concentration, suggesting an increased peripheral metabolism of Tes. Although we cannot exclude that, as previously proposed by others, the increase in Adiol-G is due to elevated metabolism of circulating DHEAS, at least in women, our data on primary cultures of RA synovial macrophages support the concept that CSA mostly increases Tes transformation into DHT [34, 35]. In addition, several studies have shown significantly lower levels of plasma (DHEAS) dehydroepiandrosterone sulphate in RA patients, particularly in women, suggesting a reduced

**TABLE III**

<table>
<thead>
<tr>
<th></th>
<th>Tes</th>
<th>DHT</th>
<th>Δ4-Ad</th>
<th>5α-Ad</th>
<th>A</th>
<th>Epi-A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6282 ± 106</td>
<td>60 ± 1</td>
<td>345 ± 7</td>
<td>6 ± 2</td>
<td>4 ± 2</td>
<td>13 ± 3</td>
<td>7250 ± 109</td>
</tr>
<tr>
<td>CSA (100 ng/ml)</td>
<td>7276 ± 10</td>
<td>84 ± 1</td>
<td>370 ± 3</td>
<td>3 ± 2</td>
<td>11 ± 2</td>
<td>24 ± 1</td>
<td>7759 ± 12</td>
</tr>
<tr>
<td>(P)</td>
<td>(0.002)</td>
<td>(0.0008)</td>
<td>(0.005)</td>
<td>(0.01)</td>
<td>(0.004)</td>
<td></td>
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<tr>
<td>CSA (500 ng/ml)</td>
<td>6113 ± 80</td>
<td>68 ± 3</td>
<td>333 ± 6</td>
<td>9 ± 2</td>
<td>5 ± 1</td>
<td>15 ± 2</td>
<td>6538 ± 81</td>
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<tr>
<td>(P)</td>
<td>(0.0008)</td>
<td>(0.01)</td>
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</table>

**TABLE IV**

<table>
<thead>
<tr>
<th></th>
<th>Tes</th>
<th>DHT</th>
<th>Δ4-Ad</th>
<th>5α-Ad</th>
<th>A</th>
<th>Epi-A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6501 ± 54</td>
<td>99 ± 1</td>
<td>322 ± 31</td>
<td>7 ± 3</td>
<td>10 ± 1</td>
<td>15 ± 4</td>
<td>6954 ± 53</td>
</tr>
<tr>
<td>CSA (100 ng/ml)</td>
<td>6933 ± 178</td>
<td>111 ± 1</td>
<td>392 ± 15</td>
<td>6 ± 3</td>
<td>12 ± 1</td>
<td>19 ± 4</td>
<td>7474 ± 196</td>
</tr>
<tr>
<td>(P)</td>
<td>(0.0001)</td>
<td>(0.02)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CSA (500 ng/ml)</td>
<td>6521 ± 16</td>
<td>114 ± 1</td>
<td>366 ± 18</td>
<td>12 ± 4</td>
<td>9 ± 3</td>
<td>15 ± 3</td>
<td>7037 ± 122</td>
</tr>
<tr>
<td>(P)</td>
<td>(0.0005)</td>
<td></td>
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</tbody>
</table>

**Testosterone metabolism in primary cultures of synovial macrophages**

Overall results from in vitro experiments on Tes metabolism by cultured RA synovial Mϕ are reported in Tables III and IV. These data indicate that CSA treatment, even in the short term (24 h), significantly affected the 17β-hydroxysteroid dehydrogenase (17βHSD) oxidative activity, which presides over the conversion of Tes into Δ4-Ad. At the same time, CSA also increased the 5α-reductase activity, which presides over conversion of Tes into DHT.

DHT formation was stimulated significantly by both 100 and 500 ng/ml of CSA after 24 h incubation (30 and 25% above control, respectively); on the other
role for the DHEAS-related activities in these patients [36, 37]. The results from in vitro studies clearly indicate that cultured RA synovial Mφ from these patients are endowed with key enzyme activity of steroid metabolism and that CSA may have a role in the regulation of androgen metabolic pathways in these cells. The evidence that the CSA-induced increase in DHT production is obtained using pharmacological CSA concentrations and short-term exposure strongly supports the view that this effect may be biologically important. This assumption is also further substantiated by the appearance in CSA-treated cells of Adiol (though in small amounts), which represents a typical DHT derivative in androgen-responsive tissues, including the prostate [38, 39].

Both Tes and DHT may exert suppressive activities on androgen receptor-positive cells involved in the immune response [40, 41]. Our results may suggest that the previously reported immunosuppressive activity exerted by CSA on an earlier stage of the immune response, i.e. antigen presentation by macrophages and by other APCs to T lymphocytes, should, at least partially, be associated with the activation, induced by the drug, of the peripheral androgen metabolism at the level of these hormone-responsive cells [16–19, 42]. In addition, preliminary results from the present study have shown a dose- and time-dependent reduction (24 and 48 h) of granulocyte-macrophage colony-stimulating factor (GM-CSF) production in conditioned media of cultured RA synovial Mφ in the presence of both Tes and CSA (data not shown). Since the GM-CSF is a macrophage activator and, among other effects, causes release of tumour necrosis factor-α (TNF-α), induction of major histocompatibility (MHC) class II molecules and potentiation of antigen processing, the observed inhibition of GM-CSF by CSA should further explain, at least partially, some of the immunosuppressive mechanisms exerted by the drug [43, 44]. As a matter of fact, a reduction of TNF-α synthesis in the presence of CSA has already been reported [45].

Although the major actions of CSA are considered to be on T lymphocytes, there is evidence for possible direct effects on other cell types, e.g. B cells, macrophages, as well as bone and cartilage cells [46]. All these cells produce a range of cytokines and are of interest in relation to the tissue changes that occur in inflammatory diseases such as RA. Therefore, the observed reduction of interleukin (IL)-1 and GM-CSF production in cultured macrophages in the presence of CSA could, in turn, influence the reduced IL-2 synthesis by T lymphocytes, already observed during CSA therapy [46]. In fact, the initial stimulus for the IL-2 production from T cells is the exposure to IL-1, derived from macrophages during the process of antigen processing and presentation.

Furthermore, APCs such as synovial Mφ contain significant levels of androgen receptors, indicating that they are potentially target cells for androgen action [12, 47]. As a matter of fact, CSA exerts immunosuppressive effects among different cells involved in the immune response, and in particular both Tes and CSA have been found to inhibit the APCs [11, 13, 16–19, 48–53]. Moreover, in recent studies, CSA was shown to increase the hepatic cytosolic androgen receptor levels in treated animals, and in another study the presence of Tes significantly prolonged the survival time of allogenic skin graft in rats treated with CSA by enhancing the immunosuppressive activity [54–56].

Since almost all the patients entered in the present CSA study experienced the ‘side-effect’ of a low-degree hypertrichosis after a mean period of 5–8 weeks, the concomitant increase in Adiol-G, in agreement with previous reports, might be confirmed as a plasma marker of the CSA activity on peripheral androgen metabolism (mainly increasing the 5α-reductase activity) [9]. Adiol-G, in fact, is not secreted by the adrenals, ovaries or testes.

The observed increase in plasma Adiol-G does not seem related to the decrease in urinary Adiol-G clearance that is a possible consequence of a reduction in the glomerular filtration rate described in CSA therapy [9]. In fact, the urinary Adiol-G clearance in CSA-treated patients was found to be greater than that observed among normal subjects [9]. Furthermore, in CSA-treated patients, no increase in the main androgens and a decrease in sex hormone binding globulin were observed, thus probably eliminating increased free Tes as the source of the reported raised Adiol-G levels [9].

Conversely, the 17βHSD activity (oxidative activity) appears to be less susceptible to CSA stimulation. Although the rise in 17βHSD activity may also lead, if prolonged, to a reduction of serum Tes, it seems more likely to postulate that the CSA-induced increase of Tes conversion to DHT at target tissue level could be mainly responsible for the influence exerted by androgens on the cells. Furthermore, the significant increase in the Tes conversion to DHT on target cells (i.e. macrophages), observed in our study, at both 100 and 500 ng/ml of CSA, seems to be in agreement with previous studies showing a biphasic pattern of inhibition of dendritic cells (APCs) pulsed with varying concentrations of the drug (from 0 to 500 ng/ml) [4].

A significant clinical improvement of the articular symptoms complained of by RA patients was observed during the CSA treatment. The effects of CSA on clinical measures in RA have already been demonstrated in several controlled trials [33, 57–59]. CSA differs from other disease-modifying anti-rheumatic drugs regarding its limited efficacy on acute-phase reactants, as confirmed by our study [33, 41, 42, 58, 59]. The implications of this for predicting impact upon the radiological progression of disease are unclear and should be a matter of future research, in relation to the reported CSA-induced influence on some RA synovial Mφ activities [47].

In conclusion, the CSA-induced hypertrichosis observed in treated patients is accompanied by an increase in peripheral androgen metabolism (i.e. Adiol-G formation). Studies in vitro on Tes metabolism by primary cultures of CSA-treated RA synovial Mφ confirm a peculiar increase in the 5α-reductase activity,
forming DHT and possibly its important metabolite Adiol.

However, further studies are needed to investigate the mechanisms of CSA action on APCs such as Mφ, by considering that CSA is generally indicated to produce immunosuppression through its inhibitory effect on T-helper/inducer and cytotoxic cell activation [60–62].

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