Discontinuous low-dose flutamide–metformin plus an oral or a transdermal contraceptive in patients with hyperinsulinaemic hyperandrogenism: normalizing effects on CRP, TNF-α and the neutrophil/lymphocyte ratio

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BACKGROUND: Low-dose flutamide–metformin (Flu-Met) with an oral contraceptive is a therapeutic option for women with hyperinsulinaemic hyperandrogenism. We questioned (i) whether Flu-Met maintains efficacy if given discontinuously; (ii) how the efficacy of discontinuous Flu-Met plus a transdermal contraceptive compares with Flu-Met plus oral contraceptive; and (iii) whether these treatments also lower circulating C-reactive protein (CRP) and tumour necrosis factor α (TNF-α) and the high neutrophil/lymphocyte ratio. METHODS: Non-obese, young patients (n = 31) with hyperinsulinaemic hyperandrogenism were started on Flu-Met (21/28 days) and randomized to receive in addition either a drospirenone oral contraceptive or a transdermal contraceptive for 6 months. RESULTS: The effects of Flu-Met were similar whether combined with oral or transdermal contraceptive. In both groups, CRP and TNF-α levels fell and the high neutrophil/lymphocyte ratio normalized (P < 0.001). Lean body mass increased (P < 0.001) in both groups but, in contrast to earlier experience with continuous Flu-Met, fat mass failed to decrease in either group. CONCLUSIONS: Flu-Met seems less lipolytic, if given for only 21 days in every 28-day period. The efficacy of Flu-Met is comparable when combined with an oral contraceptive or a transdermal contraceptive. The range of Flu-Met effects may henceforth include lower levels of CRP and TNF-α, and a normalization of the neutrophil/lymphocyte ratio.

Key words: contraception/CRP/flutamide–metformin treatment/hyperinsulinaemic hyperandrogenism/PCOS

Introduction

Polycystic ovary syndrome (PCOS), a variable constellation of anovulatory hyperandrogenism with hyperinsulinaemia and/or dyslipidaemia, is the most frequent endocrine–metabolic disorder of young women (Asunción et al., 2000; Dunaif and Thomas, 2000; Baumann and Rosenfield, 2002). The physical stigmata of women with PCOS, even if non-obese, include an excess of central fat and a deficit of lean mass, both of which seem to reflect the prolonged dysadipocytokinaemia and low-grade inflammation that accompany hyperinsulinaemic hyperandrogenism, and that are aggravated by monotherapy with an oral contraceptive (OC), even with an OC containing drospirenone (Kirchengast and Huber, 2001; Ibáñez and de Zegher, 2003, 2004a; Ibáñez et al., 2003a, 2005b; Morin-Papunen et al., 2003a).

At present, there is no approved therapy for PCOS. The addition of low-dose flutamide–metformin (Flu-Met) to a drospirenone-containing OC was recently found not only to safely reduce the fat excess and to diminish the deficit in lean mass in women with PCOS, but also to attenuate the low-grade inflammation, as judged by interleukin-6 (IL-6), adiponectin, and neutrophil count (Ibáñez et al., 2004, 2005a, b; Ibáñez and de Zegher, 2004a, b, 2005).

Tumour necrosis factor α (TNF-α) is another proinflammatory cytokine whose concentration is known to be raised in the circulation of women with PCOS, even if non-obese (González et al., 1999; Esco-bar-Morreale et al., 2001). TNF-α hyperexpression in adipose and muscle tissue has even been proposed as one of the mechanisms mediating the development of insulin resistance, through attenuation of the tyrosine kinase activity of the insulin receptor (Hotamisligil et al., 1996).

C-reactive protein (CRP) is an inflammatory marker that has recently been related to both insulin resistance and endothelial dysfunction in young and non-obese women with PCOS (Kelly et al., 2001; Tarkun et al., 2004). The high CRP levels in women with PCOS are further augmented by monotherapy with an OC, even if the OC contains an anti-androgen (Morin-Papunen et al., 2003a). Circulating CRP and low-density lipoprotein (LDL)/high-density lipoprotein (HDL)-cholesterol are increasingly recognized as two major markers of long-term cardiovascular risk (Nissен et al., 2005; Ridker et al., 2005). In
post-menopausal women, oral estrogen administration raises circulating CRP more than transdermal estrogen delivery (Lacut et al., 2003; Ropponen et al., 2005a).

Here, we questioned (i) whether Flu-Met maintains efficacy when the dose is further reduced by switching to a discontinuous (21/28 day) regimen; (ii) how the efficacy of discontinuous Flu-Met plus a transdermal contraceptive (TC) compares with Flu-Met plus a drospirenone-containing oral contraceptive (OC); and (iii) whether the beneficial effects of Flu-Met plus a contraceptive on the low-grade proinflammatory state of PCOS include also a lowering of the circulating CRP and TNF-α levels and of the high neutrophil/lymphocyte ratio.

**Study population and methods**

**Subjects**
The study population consisted of 31 young patients with hyperinsulinaemic hyperandrogenism (mean ± SEM; age 16.4 ± 0.3 yr; range 13–21 yr; body mass index 22.1 ± 0.4 kg/m²; range 18.0–25.9 kg/m²; 2–8 years post-menarche).

Inclusion criteria were (i) hyperinsulinaemia on a standard 2-h oral glucose tolerance test, defined as peak serum insulin levels >150 μU/ml and/or mean serum insulin >84 μU/ml (Vidal-Puig and Moller, 1977; Ibáñez and de Zegher, 2004a); (ii) ovarian hyperandrogenism as defined by (a) amenorrhoea (absence of menses for >3 months) or oligomenorrhoea (intermenstrual phase >45 days) or hirsutism (Ferriman–Gallwey score >8) (Ferriman and Gallwey, 1961); and (b) high serum androstenedione, total testosterone or free androgen index (testosterone × 100/sex hormone-binding globulin (SHBG)) (Ibáñez and de Zegher, 2004a, b); and (c) a 17-hydroxyprogesterone hyperresponse (>160 ng/dl) to GnRH agonist (leuprolide acetate, Procrin, Abbott, Spain, 500 μg subcutaneously) (Ibáñez and de Zegher, 2004a, b).

Prior to study start, none of the patients had received a contraceptive or another medication known to affect gonadal or adrenal function or carbohydrate or lipid metabolism for at least 9 months.

Exclusion criteria were a BMI ≥26 kg/m²; evidence of thyroid dysfunction, Cushing’s syndrome or hyperprolactinaemia; glucose intolerance (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997); family or personal history of diabetes mellitus; late-onset congenital adrenal hyperplasia (New et al., 1983; Mermejo et al., 2005); anaemia; abnormal serum electrolytes; and abnormal screening results for liver and kidney function.

The study was conducted in Barcelona without support from the pharmaceutical industry. The protocol was approved by the Institutional Review Board of Sant Joan University Hospital, and informed consent was obtained from the patients and/or their parents, with assent from minors. None of the patients or the results in the present study have been reported previously.

**Study design**

In this open-label study, all participants started on metformin (850 mg/day) and flutamide (62.5 mg) once daily (for 21 days in every 28-day period) at dinner time for 6 months. Patients were randomized [1:1 ratio; Gran Mos programme, Medical Research Institute of Barcelona (Ibáñez et al., 2004; Ibáñez and de Zegher, 2004a)] to receive, in addition, a monophasic fourth-generation OC (Yasmin, Schering; ethinylestradiol 30 μg + drospirenone 3 mg, 21/28 days; Flu-Met + OC; n = 15), or a TC containing ethinylestradiol 600 μg + norelgestromin 6 mg per weekly patch (Eva, Janssen-Cilag, 21/28 days; Flu-Met + TC; n = 16) for 6 months. In each treatment group, Flu-Met was discontinued during the cyclic week off contraception.

**Endocrine-metabolic assessment**

Fasting blood glucose, leucocyte count, serum insulin, lipid profile, sex hormone-binding globulin (SHBG) and testosterone were determined at baseline and after 3 and 6 months, together with indices of hepatic and renal function, as safety variables; the results after 3 months were intermediate between those at start and after 6 months, and will therefore not be shown. Because of financial restrictions, serum TNF-α was measured only at baseline and after 3 months; CRP was measured at baseline and after 3 and 6 months (the latter results were similar); for uniformity with TNF-α, the results after 3 months will be highlighted.

Baseline assessments were performed in the follicular phase (days 3–7) or after 2 months of amenorrhoea, and the results were compared with local references for healthy post-menarcheal females of similar age (Ibáñez et al., 2003a); in addition, 19 and 28 healthy post-menarcheal adolescents and young women served as controls for serum TNF-α and CRP measurements, respectively.

**Body composition, assays and statistics**

Body composition was assessed by dual-energy X-ray absorptiometry at study start and after 3 and 6 months, with a Lunar Prodigy coupled to Lunar software (version 3.4/3.5, Lunar, Madison, WI, USA) (Ibáñez et al., 2003b). Absolute (kg) whole-body fat and lean mass were assessed, as well as fat content in the abdominal region, which was defined as the area between the dome of the diaphragm (cephalad limit) and the top of the great trochanter (caudal limit) (Taylor et al., 1998). Total irradiation dose per assessment was 0.1 mSv. Coefficients of variation (CVs) for scanning precision were 2.0 and 2.6% for fat and lean body mass (Kiebzak et al., 2000); the intra-individual CV for abdominal fat mass was 0.7%. Body composition results after 3 months were intermediate between those at the start and after 6 months, and will therefore not be shown. Body composition references were obtained from healthy volunteers matched for gender, age, height, BMI and ethnic background.

Leucocyte, neutrophil and lymphocyte counts were assessed by an automatic cell counter (ABX Pentra 120; ABX Diagnostics, Montpellier, France) (Ibáñez et al., 2005b). Serum glucose was measured by the glucose oxidase method. Immunoreactive insulin, serum testosterone, 17-hydroxyprogesterone and SHBG were assayed as described (Ibáñez and de Zegher, 2004a). TNF-α was measured by immunochemiluminescence (Immulite 2000; Diagnostic Products, Los Angeles, CA, USA), with a lower detection limit of 1.7 pg/ml. The intra-assay and interassay CVs were 3.5 and 6.5% respectively. CRP was assessed by a highly sensitive method (Architect c8000; Abbott, Wiesbaden, Germany) with a lower detection limit of 0.1 mg/l. Serum samples were stored at –20°C until assay. For uniformity, results are expressed as mean ± SEM. Two-sided t-tests were used for statistical comparisons between groups; for each variable only one comparison was performed; the significance level was set at P < 0.05.

**Results**

Table I summarizes the key findings. At the start, this non-obese population with hyperinsulinaemic hyperandrogenism and dyslipidaemia was characterized by an adipose body composition, by high TNF-α and CRP concentrations, and by a raised leucocyte count which was due to an augmented neutrophil count. Only two patients in each treatment group had regular menses, the remaining patients being oligo- or amenorrhoeic. There were no significant differences between randomized treatment groups for any of the study indices, either at baseline or during therapy.
Flutamide–metformin plus a transdermal contraceptive in PCOS

Table 1. Clinical, haematological, endocrine–metabolic, cytokine and dual-energy X-ray absorptiometry results in young patients (n = 31; mean age 16 yr) with hyperinsulinaemic hyperandrogenism

<table>
<thead>
<tr>
<th></th>
<th>Referenceb</th>
<th>Flu-Met and OC</th>
<th>Flu-Met and TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total at 0 months</td>
<td>6 months</td>
<td>Δ 0–6 months</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.5 ± 0.5</td>
<td>22.2 ± 0.7</td>
<td>22.3 ± 0.7</td>
</tr>
<tr>
<td>Ferriman–Gallwey score</td>
<td>&lt;8</td>
<td>15.8 ± 0.9</td>
<td>16.4 ± 1.5</td>
</tr>
<tr>
<td>Leukocytes (×10⁹/mm³)</td>
<td>Total</td>
<td>6.4 ± 0.1</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>3.0 ± 0.1</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Neutrophil/lymphocyte ratio</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Glucose (mmol/l)</td>
<td>4.7 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Insulin (pmol/l)</td>
<td>80 ± 5</td>
<td>101 ± 8*</td>
</tr>
<tr>
<td></td>
<td>SHBG (nmol/l)</td>
<td>66 ± 4</td>
<td>30 ± 2*</td>
</tr>
<tr>
<td></td>
<td>Testosterone (nmol/l)</td>
<td>1.1 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Triglycerides (mmol/l)</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.6 ± 0.1</td>
<td>1.3 ± 0.3**</td>
</tr>
<tr>
<td></td>
<td>LDL-cholesterol (mmol/l)</td>
<td>1.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>TNF-α (pg/ml)b</td>
<td>3.6 ± 0.2</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>CRP (mg/l)b</td>
<td>0.6 ± 0.1</td>
<td>2.2 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>Fat mass (kg)</td>
<td>14.8 ± 1.2</td>
<td>20.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Abdominal fat mass (kg)</td>
<td>2.9 ± 0.2</td>
<td>5.8 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>Lean mass (kg)</td>
<td>37.8 ± 1.0</td>
<td>33.6 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. All patients started (at 0 months) on flutamide (Flu 62.5 mg/day; 21/28 days) and metformin (Met 850 mg/day; 21/28 days) and were randomized to receive in addition either an oral contraceptive (21/28 days) containing ethinylestradiol–drospirenone [Flu-Met and OC, n = 15] or a transdermal contraceptive patch (21/28 days) containing ethinylestradiol–norelgestromin [FluMet and TC; n = 16] for 6 months. For none of the study variables was there a significant oral-versus-transdermal contraceptive difference in the change between 0 and 6 months (Δ 0–6 months).

SHBG = sex hormone-binding globulin; TNF-α = tumour necrosis factor α; CRP, C-reactive protein.

*Healthy volunteers (age ~17 years) matched for height and weight (n = 19 for TNF-α; n = 28 for CRP and n = 24 for other variables).

bAt baseline (0 months) there were no significant differences between randomized treatment groups for any of the study variables.

Results after 3 months.

*p < 0.05; **p < 0.01; ***p < 0.001 versus reference.

†p < 0.05; ††p < 0.01; †††p < 0.001 versus baseline.

Discontinuous Flu-Met therapy combined with either an OC or a TC resulted in consistently appreciable decreases in hirsutism score, testosterone, CRP and TNF-α (Figure 1), as well as in increments in HDL-cholesterol. As expected, circulating SHBG rose, and the menses became regular in all patients.

At baseline, a robust anomaly among PCOS patients was the elevated neutrophil/lymphocyte ratio (Figure 2). Between 0 and 3 months, this high ratio fell similarly in OC and TC groups from a pooled average of 1.8 ± 0.1 to 1.4 ± 0.1 (P = 0.002), this decline being essentially attributable to a fall in the neutrophil count (from 4.4 ± 0.2 to 3.5 ± 0.1; ×10⁹/mm³; P < 0.0001) while the lymphocyte count remained unaltered. Between 3 and 6 months, the ratio normalized further to 1.2 ± 0.1 but now due to a rising lymphocyte count (from 2.6 ± 0.1 to 3.0 ± 0.1; ×10⁹/mm³; P = 0.003) while the neutrophil count remained unchanged (3.5 ± 0.1; ×10⁹/mm³).

Within 6 months, lean mass increased (P < 0.001) by an average of >1 kg in both treatment groups, but fat mass failed to decrease significantly.

In each treatment group, one adolescent experienced spotting, without breakthrough bleeding, during the second month of treatment. Treatments were well tolerated; indices of hepatic and renal function remained unchanged.

Discussion

In non-obese patients with PCOS, there is increasing evidence for a close interlinkage among hyperinsulinaemic hyperandrogenism, hyperinsulinaemic hyperandrogenism before (0 months) and after 3 months of treatment with flutamide–metformin plus either an oral (n = 15) or a transdermal (n = 16) estrogen-progestogen contraceptive. Values are percentiles 10, 25, 50, 75 and 90 of pooled results from the two treatment groups (see Table 1 for the separate groups). Treatment is associated with a consistent fall (P ≤ 0.001) in CRP and TNF-α levels, especially in patients with the most abnormal values (reference means: CRP 0.6 mg/L; TNF-α 3.6 pg/ml; Table 1).
Intervention studies have shown that monotherapy with a less adipose body composition (Ibáñez and de Zegher 2003, 2004a; Ibáñez, 2003a, 2005b) suggests that discontinuous Flu-Met maintains the essence of its endocrine–metabolic and anti-inflammatory benefits (see results of lipids, glucose/insulin, androgens, neutrophils), but is less lipolytic than continuous Flu-Met. This indirect inference remains to be confirmed by a direct comparison between the effects of continuous versus discontinuous Flu-Met on body composition.

Flu-Met seems equally effective when combined with a transdermal estro-progestogen or with a drospirenone-containing OC. This equivalence came as a surprise since the OC and TC that were compared were expected to have differential effects, for example, on SHBG, androgens, CRP or body composition (Kraemer et al., 2003; Vrbikova et al., 2004; Di Carlo et al., 2004; Sites et al., 2005; Ropponen et al., 2005a, b).

Previous studies (Ibáñez and de Zegher 2004a, 2005; Ibáñez et al., 2004, 2005b) showed that Flu-Met (with or without a contraceptive) had anti-inflammatory benefits, as judged by the circulating concentrations of IL-6 and adiponectin. The range of anti-inflammatory effects of Flu-Met (plus an OC or TC) has now been extended to a lowering of circulating CRP and TNF-α. It is plausible that these lowering effects are also attributable to Flu-Met, rather than to the coadministered OC or TC, since both oral and transdermal estrogen therapy increase these two markers (Puder et al., 2002; Lacut et al., 2003).

In an earlier paper (Ibáñez et al., 2005b), we emphasized that patients with hyperinsulinaemic hyperandrogenism have a relatively high neutrophil count, which declines within 3 months on Flu-Met treatment, and have a low-normal lymphocyte count that remains unchanged during the first 3 months on Flu-Met. Here, we corroborated those findings and extended them in two directions: firstly, the neutrophil count was found not to fall any further beyond 3 months of treatment; secondly, the lymphocyte count was found to rise after 3 months so that, between 3 and 6 months on Flu-Met (plus OC or TC) the high neutrophil/lymphocyte ratio falls further to normal. In young women, the neutrophil/lymphocyte ratio may prove to be a more robust marker of hyperinsulinaemic hyperandrogenism than the absolute neutrophil or lymphocyte count. Currently, the mechanisms whereby Flu-Met gradually normalizes the neutrophil/lymphocyte ratio are poorly understood. One possibility is that, in a first step (0–3 months), the hyperneutrophilia is lowered together with the elevated levels of other inflammatory markers (as interleukin-6, CRP and TNF-α) and that, in a second step (3–6 months), the low-normal lymphocyte count is raised in an adjustment of the total leucocyte count to the less excessive hyperneutrophilia. Changes in the expression of genes like WASP (Devriendt et al., 2001) are likely to contribute to the sequential adaptations of neutrophil and lymphocyte counts. It may not be a coincidence that WASP and the androgen-receptor gene are both encoded on the X chromosome. An alternative mechanism could be an increase in lymphocyte count through flutamide-induced testosterone blockade (Messingham et al., 2001).
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