Phosphodiesterase 4 Inhibition as a Potential New Therapeutic Target in Obese Women With Polycystic Ovary Syndrome

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Context: Phosphodiesterase (PDE) enzymes, including members of PDE4, have been investigated in the regulation of endocrine and reproductive functions of ovaries. In addition, selective inhibition of PDE4 enzyme has recently been implicated in the regulation of metabolism with positive effects on glucose homeostasis and weight reduction.

Objective: The aim of this study was to evaluate whether the PDE4 inhibitor roflumilast affects body weight and hormonal and metabolic status in obese women with polycystic ovary syndrome (PCOS).

Design/Participants/Main Outcome Measures: A 12-week prospective randomized open-label study was conducted with 36 obese women with PCOS diagnosed by the National *Eunice Kennedy Shriver* Institute of Child Health and Human Development criteria that had been pretreated with metformin (MET). They were randomized to MET 1000 mg twice a day or combined treatment (COM) with MET 1000 mg twice a day and roflumilast 500 μg every day. The primary outcome was change in anthropometric measures of obesity.

Results: Thirty-one patients (aged 33.8 $\pm$ 7.4 y, twice a day 36.4 $\pm$ 5.1 kg/m^2, mean $\pm$ SD) completed the study: 16 on MET and 15 on COM. Subjects treated with COM lost on average 4.2 $\pm$ 2.8 kg compared with a 0.9 $\pm$ 2.5 kg weight gain in the MET group ($P = .025$). Body mass index decreased for 1.6 $\pm$ 1.1 kg/m^2 in COM arm compared with increase for 0.9 $\pm$ 2.4 kg/m^2 in the MET arm ($P = .046$). Visceral adipose tissue area as assessed by dual-energy x-ray absorptiometry decreased from 136.7 $\pm$ 37.8 to 121.2 $\pm$ 36.2 cm^2 in the COM arm compared with an increase from 155.3 $\pm$ 61.9 to 166.7 $\pm$ 67.2 cm^2 in the MET arm ($P = .02$). From baseline to study end, both treatment interventions resulted in a significant reduction of androstenedione ($P = .013$), free T ($P = .002$), and homeostasis model assessment for insulin resistance score ($P = .027$) and a significant increase in SHBG ($P = .024$), although the between-treatment differences of the changes have not been statistically significant yet.

Conclusion: Roflumilast added to metformin reduced body weight in obese women with PCOS, primarily due to a loss of fat mass. **(*J Clin Endocrinol Metab* 99: E1476–E1481, 2014)**

Phosphodiesterase (PDEs) enzymes have an important role in regulation of signaling pathways that are involved in the pathophysiology of polycystic ovary syndrome (PCOS) (1, 2). They modulate steroidogenesis and complex responses to proinflammatory and metabolic mediators in ovaries (1, 3). From a conceptual point of view, there are several reasons to imply the pharmacological inhibition of PDEs as a potential new multimodal target in this endocrine disorder.

The PDE4 enzyme is a member of the PDE superfamily that is highly expressed in human ovaries (4, 5). Its selective inhibition is well recognized as an efficient treatment

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Abbreviations: BID, twice a day; BMI, body mass index; COM, combined treatment; COPD, chronic obstructive pulmonary disease; GLP-1, glucagon-like peptide-1; $HOMA_{IR}$, homeostasis model assessment for insulin resistance; MET, metformin; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PDE, phosphodiesterase; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue.

of chronic inflammatory diseases, primarily chronic obstructive pulmonary disease (COPD) (6, 7). In addition, the use of roflumilast, the first drug specifically targeting PDE4, has shown positive metabolic effects on glucose homeostasis and weight reduction in newly diagnosed type 2 diabetes mellitus (T2DM) (8).

The observed beneficial metabolic outcomes of selective PDE4 inhibition by roflumilast are based on the interplay between the PDE4 and the regulation of glucagon-like peptide-1 (GLP-1), an incretin hormone with glucose- and weight-lowering properties (9). Weight loss is the priority in the treatment of obese women with PCOS but usually remain inaccessible by classic therapeutic modalities such as lifestyle therapy and metformin. Experiences with the enhancement of GLP-1 action by GLP-1 receptor agonists are very limited in this population (10, 11). We previously reported that obese women with PCOS who have not responded to standard weight-loss strategies benefit from long-acting GLP-1 receptor agonist liraglutide as an add-on therapy to metformin regarding weight loss, whereas significant improvement of hormonal and metabolic status was not demonstrated (12).

Considering the potential integral role in metabolic, inflammatory, and steroidogenic signaling pathways and the specific tissue distribution of PDE4, we conducted a pilot study powered specifically for weight loss in PCOS.

The study is registered (www.clinicaltrials.gov) as identification number NCT02037672.

Materials and Methods

Study design

A 12-week prospective, randomized, open-label study was conducted with 36 obese women with PCOS diagnosed by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development criteria that had been pretreated with metformin (MET) due to metabolic disturbances or anovulation. They were randomized to MET 1000 mg twice a day (BID) or combined treatment with roflumilast 500 μg every day added to MET 1000 mg BID (COM). A diet of 500–800 kcal/d reduction made up of 50% carbohydrates, 20% proteins, and 30% of fat with increased consumption of fiber, whole grains, cereals, fruits, and vegetables along with at least 30 minutes of moderate intensity physical activity daily had been recommended to all women when the diagnosis of PCOS had been confirmed and metformin had been initially prescribed. At the beginning of the study, lifestyle intervention was not again actively promoted.

Patients were excluded if they had history of neuropsychiatric events or had lost or gained more than 5% of their body weight in the previous 3 months. The primary outcome was a mean change in measures of obesity. Secondary outcomes included hormonal and metabolic changes. After randomization and at study end point, all patients underwent standard anthropometric measurements: height, weight, waist circumference, blood pressure, and measurement of whole-body composition by a Hologic dual-energy x-ray absorptiometer, including total body fat and visceral adipose tissue (VAT) mass. Body mass index (BMI) was calculated as the weight in kilograms divided by square of height in meters. A fasting blood was drawn for determination of glucose, insulin, LH, FSH, androstenedione, dehydroepiandrosterone sulfate, and total and free T followed by a standard 75-g oral glucose tolerance test (OGTT) to assess glucose homeostasis. Impaired glucose tolerance was identified by 2-hour glucose levels between 7.8 and 11.0 mmol/L, as defined by the American Diabetes Association criteria. Determination of metabolic syndrome was based on the International Diabetes Federation definition (13). The calculation for homeostasis model assessment for insulin resistance ($HOMA_{IR}$) was applied as a measure for insulin resistance. Safety clinical assessment was performed at the beginning of the study and at weeks 4, 8, and 12 of the treatment period. Pregnancy was excluded by measuring β-human chorionic gonadotropin. The women were advised to strictly use barrier contraception.

Study approval

The study was approved by a National Ethical Review Committee and conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines. All patients gave written informed consent.

Statistical analysis

The primary end point of this study, for which a power calculation was used to determine the sample size, was a mean change in weight. Given that there were no previous studies using roflumilast alone or combined with metformin treatment for PCOS, we based our calculations on a previous study with comparator combined treatment intervention (12). It was determined that 14 patients would be needed to give a power of 80% to detect statistically significant difference ($\alpha = .01$) of approximately 2.5% in weight loss and estimated dropout rate of 15%. The power estimate was performed on the online calculator at http://hedwig.mgh.harvard.edu/sample_size/size.html.

Results are presented as mean ± SD. Normal data distribution was checked with the Shapiro-Wilk test. Differences at baseline between the treatment groups were checked with general linear model. The differences between treatment groups were checked and confirmed using a repeated-measures *t* test. Treatment impact on the primary outcome measure was analyzed with evaluable patient data using a repeated-measures, linear mixed-effects model with time points (baseline, 12 wk) and therapeutic groups (MET arm, COM arm). Multiple testing of secondary outcomes was not performed. A value of $P < .05$ was considered statistically significant. Other than sample size calculation, all statistical analyses were performed using the SPSS 17.0 Statistical Software Package.

Results

Baseline results

The study enrolled 36 participants. In the first 2 weeks, one patient treated with COM discontinued the study because of headache, one because of nausea, and one because of abdominal pain. Two patients were excluded from the MET group due to protocol violation in the last month of

the study. Thirty-one patients (aged 33.8 ± 7.4 y, BMI 36.4 ± 5.1 kg/m^2, mean ± SD) completed the study: 16 on MET and 15 on COM. There were no significant differences at baseline in any of the parameters between the treatment groups. Baseline characteristics of the study outcomes are provided in Tables 1 and 2.

Measures of obesity

There was a significant differential effect of COM compared with MET with respect to weight, BMI, total body fat, and VAT area. At study end point, subjects treated with COM lost on average 4.2 ± 2.8 kg compared with a 0.9 ± 2.5 kg weight gain in MET group ($P < .001$). BMI decreased for 1.6 ± 1.1 kg/m^2 in COM arm compared with an increase for 0.9 ± 2.4 kg/m^2 in the MET arm ($P = .001$). Total body fat decreased for 0.7% ± 0.4% in COM as opposed to 0.2% ± 0.1% increase in MET, and VAT area decreased for 15.5 ± 1.6 cm^2 in COM as opposed to an 11.4 ± 5.3 cm^2 increase in MET. The greater waist circumference reduction was noted in COM (4.2 ± 1 cm) compared with MET (0.8 ± 0.7 cm). However, the between-treatment difference was not statistically significant. The mean pre- and posttreatment measures of obesity are presented in Table 1.

Metabolic parameters

Five women in the MET and three in the COM group had impaired glucose tolerance at the beginning of the study. Treatment intervention resulted in normal glucose homeostasis in 75% of the women, in four of five in the MET and two of three in the COM group. At baseline metabolic syndrome was present in eight women in the MET and eight in the COM group. At the end of the observed period, the metabolic syndrome resolved in three of eight patients in the COM group and persisted in all patients in the MET group. $HOMA_{IR}$ was unchanged in the MET and decreased for 0.7 in the COM group, although the between-treatment difference was not statistically significant yet. There was a statistically significant within-treatment change from baseline to the last visit in fasting glucose levels. The mean pre- and posttreatment values of the metabolic parameters are presented in Table 2.

Endocrine parameters

At 12 weeks a significant androstenedione and free T reduction and SHBG increase were noted in both treatment groups when compared with baseline. Free T reduction and SHBG increase were shown as being slightly greater in patients treated with roflumilast and MET combination (−2.09 ± 3.02 nmol/L, 5.53 ± 9.80 nmol/L, respectively) compared with MET monotherapy (−1.21 ± 2.07 nmol/L, 1.21 ± 8.82 nmol/L, respectively). However, the between-treatment differences were not statistically significant (Table 2). No statistically significant within- and between-treatment differences were found in total T, dehydroepiandrosterone sulfate, LH, and FSH.

Changes in menstrual pattern

Menstrual frequency did not change in the MET group (0.8 ± 0.34 to 0.8 ± 0.28) and increased from 0.83 ± 0.31 to 0.87 ± 0.21 per month in the COM group. No statistically significant changes were found, neither over time nor when analyzing it separately by therapeutic arm.

Adverse events

Adverse events in evaluable patients receiving roflumilast were mild or moderate. The most commonly reported adverse events in the COM group were diarrhea (3 of 15), nausea (3 of 15), and headache (1 of 15). All reported adverse events resolved within 4 weeks of the onset. There was no report of a neuropsychiatric event. No adverse events were reported in the MET group.

Discussion

This is the first report demonstrating that the selective PDE 4 inhibitor roflumilast added to MET was superior to MET alone in reducing mean body weight after 12 weeks

Table 1. Baseline and 12-Week Posttreatment Measures of Obesity

	MET (n = 16)		COM (n = 15)		
	Baseline	After Therapy	Baseline	After Therapy	*P* Values
Weight, kg	105 ± 11.1	105.9 ± 11.2	98.3 ± 16.7	94.1 ± 16.2	T = .000; I = .025
BMI, kg/m^2	37.1 ± 4.3	38 ± 5.4	35.8 ± 5.8	34.2 ± 5.3	T = .002; I = .046
Waist circumference, cm	120.7 ± 12.2	119.9 ± 11.5	115.3 ± 13	111.1 ± 14	T = .000
Total body fat, %	44.1 ± 3.8	44.3 ± 3.9	42.5 ± 4.5	41.8 ± 4.1	T = .032
VAT area, cm^2	155.3 ± 61.9	166.7 ± 67.2	136.7 ± 37.8	121.2 ± 36.2	I = .020

Abbreviations: I, interaction differences between treatment over trials; T, overall effect after all treatments.

Table 2. Baseline and 12-Week Posttreatment Metabolic and Endocrine Parameters

	MET (n = 16)		COM (n = 15)		
	Baseline	After Therapy	Baseline	After Therapy	*P* Values
Glu 0 min OGTT, mmol/L	4.9 ± 0.9	4.7 ± 0.7	5.1 ± 0.5	4.8 ± 0.4	T = .038
Insulin 0 min OGTT, mU/L	14.5 ± 10.1	13.4 ± 8.1	12.2 ± 8.7	9.7 ± 6	NS
HOMA-IR	3 ± 2.4	3 ± 2.1	2.8 ± 2.2	2.1 ± 1.3	T = .027
Total T, nmol/L	1.5 ± 1.1	1.6 ± 1	1.3 ± 0.6	1.5 ± 0.5	NS
SHBG, nmol/L	37.1 ± 37.2	38.3 ± 30.3	32.7 ± 18	38.2 ± 25.6	T = .024
Free T, nmol/L	4.5 ± 3.8	3.3 ± 3.1	4.2 ± 3.3	2.1 ± 1.4	T = .002
Androstenedione, nmol/L	10.9 ± 6.2	7.6 ± 3	10 ± 4.8	6.9 ± 2.2	T = .013
DHEA-S, μmol/L	6 ± 3.8	6.6 ± 3.9	6.6 ± 2.2	7.3 ± 3.2	NS

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; Glu, glucose; I, interaction differences between treatment over trials; NS, not significant; T, overall effect after all treatments.

of treatment in obese women with PCOS, primarily due to a loss of fat mass. The improvements of obesity measures were associated with beneficial effects on fasting glucose levels, insulin resistance, and resolution of metabolic syndrome in affected women in the COM group. In addition, the decrease of free T and the increase of SHBG tended to be greater in patients treated with a combination of roflumilast and MET compared with MET monotherapy.

In a pooled analysis of the two 12-month, placebo-controlled studies in patients with COPD, roflumilast was associated with a weight decrease of about 2 kg. When stratified by the presence of T2DM, weight loss in patients with COPD and T2DM treated with roflumilast was 2.69 kg, compared with 1.99 kg in patients with COPD and without T2DM (6, 7). In a 12-week, randomized, double-blind, placebo-controlled study of patients with newly diagnosed T2DM without COPD, the roflumilast therapy reduced a mean weight change of −1.9 kg. However, because the study consisted of patients with newly diagnosed T2DM who had received advice on lifestyle intervention, the placebo group also lost 1.2 kg and the difference between treatment groups was not statistically significant (8). Most of the weight decrease in COPD patients treated with roflumilast occurred in the first 6 months of treatment (6, 7). The hypothesis behind the weight decrease observed in roflumilast is based on the PDE4 regulation of signaling pathways linked to GLP-1 release. In an experimental rodent model, a single treatment with 10 mg/kg roflumilast enhanced plasma GLP-1 levels up to 2.5-fold (9).

In the present study, patients on roflumilast added to MET lost on average 4.2 kg in comparison with MET group that gained on average 0.9 kg, with the between-treatment difference around 5 kg. The observed effects of roflumilast added to MET on body weight in PCOS were expectedly similar to the effects of the GLP-1 receptor agonist added to MET in PCOS. We previously demonstrated that subjects with PCOS of comparable age and BMI treated with the long-acting GLP-1 receptor agonist liraglutide that was added to MET lost on average 6.5 kg compared with a 1.2-kg loss in the MET group, with the between-treatment difference of 5.3 kg, achieved in the same treatment period of 12 weeks (12). Furthermore, the combined treatment with the short-acting GLP-1 receptor agonist exenatide and MET resulted in a mean weight loss of 6 kg compared with a mean loss of 1.6 kg in the MET group, with the between-treatment difference of 4.4 kg achieved in 24 weeks (8). When compared with the effect of roflumilast on body weight in patients with COPD and T2DM, the mean reduction of body weight in our patients with PCOS was greater, suggesting previously reported correlation between BMI and weight loss. In patients with COPD treated with roflumilast, the highest proportion of subjects who experienced weight decrease and the largest absolute weight decrease were observed mainly in the obese patients, followed by patients who were classified as overweight (6). In addition, MET in combination with roflumilast may exert its additive beneficial action in part through the modulation of the incretin axis by the stimulatory effect of GLP-1 (14).

Bioimpedance measurements in patients with COPD indicated that the weight decrease associated with roflumilast was primarily due to reduction of fat mass (7, 8). Similarly, we observed the potentially beneficial effects of roflumilast on body composition in our patients. The mean difference of 3.4 cm of waist circumference was observed and in favor to the COM group. In addition, total body fat and VAT area decreased, whereas both these parameters slightly increased in MET monotherapy. Recognizing that increased amounts of adipocytes are associated with numerous abnormalities of sex steroid metabolism such as increased androgen production and suppression of SHBG, the reduction of visceral fat in particular would be a highly desired outcome in PCOS.

As prespecified secondary outcomes, we observed greater mean $HOMA_{IR}$ score reduction in patients treated with roflumilast as compared with the MET group in the observed treatment period. Furthermore, the COM treatment has also been more successful in the reversibility of fully blown metabolic syndrome when compared with MET monotherapy. The obtained positive within-treatment effect on fasting glucose was in line with the results in the roflumilast group in patients with T2DM. The beneficial impact of roflumilast on metabolic parameters has been explained by the same mechanisms mediated through its elevating effect on the GLP-1 incretin hormone levels as being hypothesized to be responsible for its effect on body weight reduction (8).

Androstenedione and free T levels were significantly decreased, whereas the SHBG was significantly increased in both treated groups, noticing that the improvement of free T and SHBG tended to be slightly greater in patients treated with roflumilast added to MET. Interestingly, no improvement in free T and SHBG were observed with liraglutide added to MET despite the comparable effect of both combined treatments on the weight reduction in obese women with PCOS (12). Due to the generally known methodological problem for assessment of androgens in PCOS, relatively small study sample size, and lack of any data on roflumilast specific effects on androgen levels, we currently cannot provide any firm conclusion about this observation. The observed trend could definitely be related to the significant weight loss in the COM group. In addition, the expression of PDE4 enzymes in ovaries and the recognized involvement of PDEs in steroidogenesis do not exclude a potential role of roflumilast in direct regulation of ovarian steroidogenesis and in the pathophysiology of PCOS.

The present study has several limitations. It was not designed as a double-blind, placebo-controlled study and was performed in a small population that had been pretreated with metformin. We recently conducted an ongoing 24-week, prospective, randomized, open-label study with treatment-naïve obese women with PCOS randomized to MET 1000 mg BID or roflumilast 500 μg every day to further investigated the potential clinical efficacy of roflumilast in PCOS. Other larger and better-designed trials of longer duration as well as studies designed to investigate the possible mechanisms by which roflumilast might be involved in the pathophysiology of PCOS are warranted to assess the efficacy and safety of roflumilast in this population. However, the main strength of our study is the original concept that grasps selective PDE4 inhibition in obese women with PCOS as a new therapeutic target based on the specific tissue distribution and functional properties of PDE4 enzymes and the reported metabolic outcomes of selective PDE4 inhibitor roflumilast in COPD and T2DM populations.

In conclusion, roflumilast added to metformin reduced body weight in obese women with PCOS, primarily due to a loss of fat mass. Our observations are not definitive but do encourage further exploration of the use of this class of medications in metabolic conditions associated with obesity. The drug's precise mechanism of action in PCOS remains to be elucidated.

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