Berberine reduces insulin resistance induced by dexamethasone in theca cells in vitro

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, insulin resistance (IR), and progression to type 2 diabetes (1). As demonstrated by Dunaif et al. (2–4) in studies of the mechanism of PCOS and metabolic phenotype in women with the syndrome, IR has a pivotal role in the pathogenesis of ovarian androgen excess. We have shown that IR exists within PCOS ovaries and is caused by defective insulin signaling (5, 6), indicating a direct association between androgens and IR within ovary theca cells. In primary cultured rat adipocytes, dexamethasone impairs insulin signaling and glucose transport by depleting insulin receptor substrate 1 (IRS-1) and phosphatidylinositol 3-kinase (7, 8). To investigate the association between IR and hyperandrogenism in PCOS, we established an in vitro model of dexamethasone-induced IR in ovarian theca cells. We also investigated the effects of berberine, which is one of the main constituents of *Rhizoma coptidis* (9) and has been used to treat both diabetes mellitus and PCOS in traditional Chinese medicine.

The study was approved by the Institutional Review Board of Heilongjiang University of Chinese Medicine, Harbin. Porcine ovaries were obtained from a slaughterhouse, maintained in warm 0.9% sodium chloride solution, and transported to the laboratory within 2 hours. Theca-granulosa membranes were removed from immature follicles (6–9 mm), and granulosa cells were detached by mechanical separation. Residual tissue was cut into small pieces (2–3 mm) and cultured in Ham F-12/Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum for 2 days. Theca cells expanded from the fringe of the tissue pieces and became more confluent. At 4 days, these cells were detached with trypsin (0.25 g/L; Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China), and the cell suspension was collected, placed into culture flasks (Orange Scientific Company, Braine-l’Alleud, Belgium) in Ham F-12/Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum for 2 days. Theca cells expanded from the fringe of the tissue pieces and became confluent. At 4 days, these cells were detached with trypsin (0.25 g/L; Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China), and the cell suspension was collected, placed into culture flasks (Orange Scientific Company, Braine-l’Alleud, Belgium) in Ham F-12/DMEM with 10% fetal calf serum for 2 days.

Attached theca cells were exposed to Ham F-12/DMEM containing with 4% bovine serum albumin Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China and 10 nmol/L insulin Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China with or without 1 µmol/L dexamethasone Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China as previously described (7) to induce IR. After 48 hours, the medium of IR group was replaced with Ham F-12/DMEM containing 4% bovine serum albumin and 10 nmol/L insulin, and berberine (1.5 µmol/L) or vehicle was added to the medium. All compounds were dissolved in dimethyl sulfoxide.

Received March 29, 2010; revised July 10, 2010; accepted July 30, 2010; published online September 16, 2010.

L.Z. has nothing to disclose. W.L. has nothing to disclose. F.H. has nothing to disclose. J-P.B. has nothing to disclose. X.W. has nothing to disclose. L.H. has nothing to disclose. H.K. has nothing to disclose. Y.W. has nothing to disclose. F.H. has no other financial relationships that may be relevant to the submitted work.

Supported by the National Clinical Research Base of Gynecology in China, Heilongjiang Province Foundation for Outstanding Youths (JC200804).

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sulfoxide and applied to the cells in a volume equal to 0.1% of the medium. After 72 hours, the culture medium was collected for glucose and steroid assays, and cells were collected for assessment of mRNA expression of target genes.

Progestin and testosterone levels were determined with standard radioimmunoassay kits (Beijing Furui Bio-engineering Company, Beijing, China). Glucose concentration was measured with Glucose-HK kits (Sigma). To confirm IR in the theca cell model, we assessed glucose uptake. Dexamethasone-treated and control cells were incubated at 37°C in Ham F-12/DME (0.86 mmol/L) with 4% bovine serum albumin and 1 μCi/well [3H]glucose (Amersham Pharmacia) in the presence of insulin (0.1 μmol/L). After 2 hours, the cells were collected, and [3H]glucose uptake for glycogen synthesis was measured. IR was assessed by determining the difference in insulin-stimulated glucose uptake between the two groups.

Total RNA was extracted from cells with Trizol reagents (Invitrogen, Carlsbad, CA). RNA was assayed with the Thermo Script RT-PCR System (Invitrogen, Carlsbad, CA). PCR reactions were performed with a thermal cycler (Eppendorf, Hamburg, Germany). The amplification process and the primer sequences for target genes are described elsewhere (10, 11).

All experimental data are presented as mean ± SEM. Results are representative of at least three independent experiments. Differences were determined by analysis of variance and Tukey post hoc test; P < .05 was considered to be statistically significant.

Insulin-stimulated [3H]glucose uptake was nearly 2-fold greater in control than in dexamethasone-treated cells (P < .01), confirming that dexamethasone impairs the action of insulin in our model of theca cell IR. The residual glucose level in the medium was ~30% higher in dexamethasone-treated IR theca cells than in control cells (P < .01). Residual glucose levels were also lower in the medium of berberine-treated cells than in the medium of IR theca cells (P < .05), indicating that berberine improves the action of insulin.

Next, we examined the mRNA expression of three key molecules of the insulin signaling cascade (Fig. 1). IRS-1 expression was 49% lower (P < .05), glucose transporter 4 (Glut-4) expression was 73.8% lower (P < .05), and peroxisome proliferator–activated receptor γ (PPAR-γ) expression was 45.4% higher (P < .01) in IR thecal cells than in control cells. This expression pattern was partially reversed in IR cells treated with berberine (Ber). Results are representative of at least three independent experiments. (Bottom) Gene expression was determined by the intensity of ethidium bromide × area/β-actin × area and expressed in arbitrary units. Values are mean ± SEM, #P < .01 vs. control group; ##P < .05 vs. IR group. GSR = gray scale ratio (shows the fold of target genes expression of beta-actin).

To investigate the relationship between theca IR and hyperandrogenism, we examined the levels of 17-hydroxylase (CYP17), a crucial enzyme in androgen biosynthesis, T, and P in the medium. The mean P levels were similar in the three groups, but the T level was 2-fold higher in dexamethasone-treated theca cells in the IR group than in control cells (P < .01). In addition, CYP17 mRNA expression was 10.6-fold higher in the IR group than in control cells (P < .01). In the berberine group, CYP17 mRNA expression was 62.5% lower than in the IR group (P < .05), and the T level in the medium was similar to that in the control cells.

The association between IR and hyperandrogenism is evidenced by the observation that insulin-sensitizing agents reduce both IR and androgen levels in women with PCOS (12). Pathways that control nongenomic effects of insulin appear to be vulnerable to disruption, with disparate functions of insulin affected. However, persistent insulin-mediated hyperandrogenism seems to be limited to women with PCOS and are uncommon in women with moderate obesity and noninsulin-dependent diabetes mellitus. In women, ovarian theca cells are the primary source of androgen biosynthesis (13). IR as the primary defect in the polycystic ovary may confer this susceptibility.

To investigate the effect of IR on hormonal synthesis, we used dexamethasone-treated ovarian theca cells as an in vitro model of IR. Dexamethasone significantly increased T levels and accumulation of CYP17 mRNA; however, P levels in the medium did not change significantly. CYP11A1 and CYP17 are crucial enzymes in T biosynthesis, but P is controlled by CYP11A1 and HSD3B. Dexamethasone might have two distinct effects on these key enzymes. In human choriocarcinoma JEG-3 cells, dexamethasone has little effect on the function of HSD3B (14), which may explain the lack of effect on P. Theca cells with dexamethasone-induced IR accumulated 10-fold more CYP17 mRNA than control cells, although we did not check expression of 17,20-lyase within CYP17. These results strongly suggest that IR theca cells are the source of the excess androgens in women with PCOS.

There seems to be a disparity between the metabolic and steroidogenic actions of insulin in the polycystic ovary. To explore this issue, we turned to our model of IR in ovarian androgenic
theca cells. As in rat adipocytes (7), dexamethasone significantly decreased IRS-1 and Glut-4 mRNA levels. Reduced Glut-4 mRNA content in adipose tissue has been reported for patients with type 2 diabetes (15) and could contribute to impairment of intracellular signaling cascades involved in glucose metabolism in theca cells. The glucocorticoid receptor regulates the expression of wide range of genes, including those encoding factors that regulate glucose metabolism (16). Therefore, in theca cells, dexamethasone may interact with its receptor to impair glucose uptake but promote testosterone biosynthesis—a potential explanation for the hyperandrogenism in women with PCOS. However, we cannot exclude from our data that dexamethasone directly alters hormonal synthesis in theca cells.

In traditional Chinese medicine, berberine has been prescribed to reduce IR in patients with diabetes (17, 18). Berberine may also reduce serum cholesterol, triglycerides, and low-density lipoprotein cholesterol in hypercholesterolemic patients and in animals fed a high-fat diet (19). In our IR theca cell model, berberine increased Glut-4, decreased PPAR-γ mRNA levels, increased glucose uptake, and reduced IR. All of these results indicate that berberine can improve both IR and androgenic potential in IR theca cells. Berberine decreased testosterone level in the medium and PPAR-γ mRNA expression, indicating that berberine influences the PPAR-γ cascade. Although berberine can reduce IR, the mechanisms of its effects in women with PCOS needs further investigation.

In summary, theca IR induced by dexamethasone increased androgenic potentials and was antagonized by berberine intervention, suggesting involvement of theca IR in the pathogenesis of androgen excess in PCOS.

REFERENCES