Global gene expression analysis in liver of db/db mice treated with catalpol

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[ABSTRACT] Catalpol, a major bioactive component from Rehmannia glutinosa, which has been used to treat diabetes. The present study was designed to elucidate the anti-diabetic effect and mechanism of action for catalpol in db/db mice. The db/db mice were randomly divided into six groups (10/group) according to their blood glucose levels: db/db control, metformin (positive control), and four dose levels of catalpol treatment (25, 50, 100, and 200 mg·kg⁻¹), and 10 db/m mice were used as the normal control. All the groups were administered orally for 8 weeks. The levels of fasting blood glucose (FBG), random blood glucose (RBG), insulin tolerance, and glycated serum protein (GSP) and the genome expression in liver tissues were analyzed. Our results showed that catalpol treatment obviously reduced water intake and food intake in a dose-dependent manner. Catalpol treatment also remarkably reduce fasting blood glucose (FBG) and random blood glucose (RBG) in a dose-dependent manner. The RBG-lowering effect of catalpol was better than that of metformin. Furthermore, catalpol significantly improved glucose tolerance and insulin tolerance via increasing insulin sensitivity. Catalpol treatment significantly decreased GSP level. The comparisons of gene expression in liver tissues among normal control mice, db/db mice and catalpol treated mice (200 and 100 mg·kg⁻¹) indicated that there were significant increases in the expressions of 287 genes, which were mainly involved in lipid metabolism, response to stress, energy metabolism, and cellular processes, and significant decreases in the expressions of 520 genes, which were mainly involved in cell growth, death, immune system, and response to stress. Four genes expressed differentially were linked to glucose metabolism or insulin signaling pathways, including Irs1 (insulin receptor substrate 1), Idh2 (isocitrate dehydrogenase 2 (NADP⁺), mitochondrial), G6pd2 (glucose-6-phosphate dehydrogenase 2), and SOCS3 (suppressor of cytokine signaling 3). In conclusion, catalpol exerted significant hypoglycemic effect and remarkable therapeutic effect in db/db mice via modulating various gene expressions.

[KEY WORDS] Catalpol; db/db Mice; Antidiabetic effect; DNA microarray; Gene expression; SOCS3


Introduction

Diabetes mellitus is a heterogeneous metabolic disease and one of the most serious diseases threatening human health. The disease is rapidly increasing worldwide. According to WHO statistics, the global number of diabetes patients has reached about 300 million and there are six people suffering from diabetes deaths every minute by 2025. Diabetes can lead to various complications, such as diabetic nephropathy, diabetic encephalopathy, diabetic retinopathy, diabetic foot and other complications, affecting patients’ quality of life. There are two major types of diabetes, namely type 1 and type 2. Type 1, insulin-dependent diabetes mellitus (IDDM), accounts for 5%–10% diabetes. People with type 1 diabetes must take daily insulin injections. Type 2, non-insulin-dependent diabetes mellitus (NIDDM), accounts for 90%–95% diabetes and insulin resistance is a major feature of type 2 diabetes. There are several classes of anti-diabetic medications available. Metformin is generally recommended as a first line treatment; however, it should not be used in those with severe kidney or liver problems. Thus, diabetic patients and healthcare professionals are interested in natural products with a therapeutic potential in diabetes treatment, particularly those derived from plants because they are regarded to be less toxic with fewer side effects when compared to their synthetic counterparts.
Radix Rehmannia (DI Huang), the roots of Rehmannia glutinosa Libosch., is commonly used in traditional Chinese medicine. It has been used in China to treat “Xiaoake” (diabetes) for nearly a thousand years. Catalpol, an iridoid glucoside (Fig. 1) in Rehmannia glutinosa, has been reported to have multiple biological activities, such as anti-diabetic, antioxidiant, antitumorand neuroprotective properties [11-15]. Catalpol is the main component for the anti-diabetic effects of Radix Rehmannia. The aim of the present study is to investigate the effects of catalpol in db/db mice.

**Materials and Methods**

**Materials and reagents**

Catalpol was obtained from Qinghai Yangzong Pharmaceutical Co., Ltd. (Lot#, TQ101106; purity, 95.57%); Metformin was production of Tianjin Pacific Pharmaceutical Co., Ltd. (Lot#, 070708); Novolin R biosynthetic human insulin injection was production of Novo Nordisk (USA) Co. (Lot#, XVG0253); Glucose was purchased from Tianjin Chemical Reagent Factory (Lot#, 20100325); GSP commercial kit was purchased from Nanjing Jiancheng Bioengineering Institute (Lot#, 20110418); and insulin Elisa kit was purchased from Adlitteram Diagnostic Laboratories (GBD, Lot#, RT110371).

**Instrument**

Glucometer (ACCU-CHEK Aviva) was made by Roche Diagnostics GmbH; Electronic balance (G&G T2000) was production of American twin brothers Jason (Group) Co., Ltd.; Electronic balance (PL203) is Mettler Toledo Instruments (Shanghai) Co., Ltd.; and Affymetrix GeneChip 3000 TG System was made by Affymetrix.

**Animals**

Four-week-old db/db mice and db/m mice were purchased from the Suzhou Industrial Park, Ireland Matt Technology Co., Ltd., and allowed one week for environmental acclimation. The mice were maintained under standard light (12 h/12 h light/dark cycle), controlled temperature (20–26 °C) and relative humidity (40%–70%) conditions. The mice had free access to food and water ad libitum. All the experimental procedures were performed in accordance with International Guidelines for Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Tianjin Institute of Pharmaceutical Research New Drug Evaluation Co., Ltd.

**Experiment design**

Sixty mice were selected from seventy db/db mice with FBG > 12.0 mmol·L⁻¹ and randomly divided into following six groups according to their blood glucose levels with 10 mice in each group: db/db control group, metformin group, 4 groups treated with various doses of catalpol (25, 50, 100, and 200 mg·kg⁻¹), and 10 db/m mice were used as the normal control group. All the groups were administered with the drugs or control orally for 8 weeks. Body weights were measured once per week, food intake and water intake were measured once per day, except fasting day. At the end of experimental period, the mice were fasted overnight, and liver and blood samples were collected. Liver tissues was kept in liquid nitrogen. Serum was obtained by centrifugation and frozen at −80 °C until analysis.

**Measurements of FBG and RBG**

FBG: blood samples for glucose assay were obtained from the tailveins of mice after fasting 6 h (2 h after treatment) weekly. On the 14th day of treatment, blood samples were collected at 0, 1, 2, 3, and 4 h post treatment after fasting 4 h.

RBG: blood glucose levels were measured at 0, 1, 2, 3, 4, 6, and 8 h post treatment in no fasting mice on the 28th day of treatment.

Blood glucose was measured with an Accu-check Advantage glucometer and blood glucose teststrips (Roche Diagnostics GmbH, Mannheim, Germany).

**Glucose tolerance and insulin tolerance tests**

An oral glucose challenge test was performed in mice after 12-h fasting on the 49th days of treatment. Oral glucose load was administered at 1 g/kg body weight. Blood glucose levels were measured at 0, 30, 60, and 120 min after oral gavage of glucose.

An insulin tolerance test was performed in on the 49th days of treatment. The mice were fasted for 4 h before insulin tolerance test. Blood glucose levels were tested at 0, 30, 60, 90, 120, 180, and 240 min after intraperitoneal injection of 0.5 IU·kg⁻¹ insulin.

**Determination of GSP level**

At the end of the experiment, the mice were fasted for 12 h. Blood samples were collected by orbital sinus puncture using capillary tubes. Thesea were prepared by centrifuging the blood samples at 3000 r·min⁻¹ for 10 min. Serum levels of glycated serum protein (GSP) was determined using commercial kits, following manufacturer’s instructions.

**Gene expression**

To know insight into the molecular mechanism of catalpol’s action, we performed a global gene expression profiling study using DNA microarray technology. DNA micro-array analysis used Affymetrix GeneChip and total RNA was extracted from each mouse liver with TRIZOL reagent and purified using RNEASY kit.

In brief, the mice were divided into four groups, including db/m control group, db/db control group and two groups treated with 200 or 100 mg·kg⁻¹ of catalpol. We performed DNA micro-array analysis via Affymetrix GeneChip (Mouse u430 plus 2.0) technology with total RNA prepared from the pooled samples of liver tissues, in which equal amounts of...
RNA from five individual animals from each group were mixed. And there were two pooled liver samples in each group to determine gene expression.

**Data analysis**

The data were expressed as means ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet’s post hoc test, if appropriate. A value of $P < 0.05$ was considered statistically significant.

**Results**

**Effects of catalpol on food and water intake**

As shown in Fig. 2, the food intake and water intake in db/db control group were significantly higher than that in the normal control group.

Catalpol treatment can remarkably reduce the food intake and water intake in a dose-dependent manner, compared with the model group.

![Fig. 2  Effects of catalpol on food and water intake in db/db mice. A: food intake; B: water intake. △△△ $P < 0.001$ vs normal control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs db/db control](image)

**Effects of catalpol on FBG**

As shown in Fig. 3, the fasting blood glucose levels in db/db control mice were obviously higher than that in the normal control group. No differences in FBG before administration of drugs were found among the groups except normal control. The significantly decreased mean FBG level was first seen with 200 mg·kg$^{-1}$ of catalpol treatment ($P < 0.01$). After drug administration for one week, a steady and marked reduction in the serum glucose level was observed in metformin and three catalpol groups (50, 100, and 200 mg·kg$^{-1}$) compared with the db/db control group. Moreover, on the 14th, 20th and 34th days of treatment, the serum glucose level also obviously reduced in catalpol group at the lowest test dose (25 mg·kg$^{-1}$).

Our results showed that a significant reduction in blood glucose in the metformin and two catalpol groups (100 and 200 mg·kg$^{-1}$) before the administration on the 14th day of treatment. Furthermore, the blood glucose levels were significantly reduced at 1 h after administration for all catalpol groups, and significant hypoglycemic effects were seen in the groups of 200, 100, 50 mg·kg$^{-1}$ catalpol until 4 h after administration.

**Effects of catalpol on RBG**

The non-fasting blood glucose levels of all the mice were tested on the 28th day of treatment. The blood sugar levels were dramatically reduced in metformin and all catalpol groups before the administration. Then, the blood sugar levels of mice treated with metformin and catalpol were significantly decreased and remained low until 8 h after administration. Our results showed that catalpol had better hypo-glycemic effect on RBG than metformin.

**Effects of catalpol on glucose tolerance and insulin tolerance**

An oral glucose tolerance test was performed in mice on the 43rd day of catalpol treatment. The blood glucose concentrations were elevated in all groups at 30 min after glucose administration (Fig. 4) and the blood glucose levels in the db/db mice were increased, compared with the normal control.

An obvious improvement in OGTT was observed in the metformin and three catalpol groups (50, 100, 200 mg·kg⁻¹); however, there were no statistically significant differences in blood glucose concentration between the lowest catalpol dose group (25 mg·kg⁻¹) and the db/db control groups.

Insulin tolerance test was also improved significantly, as evidenced by lower glucose levels and AUC area in the metformin and all the catalpol groups.

**Fig. 3** Effects of catalpol on fasting blood glucose in the db/db mice treated with various drugs and control. A: weekly FBG; B: FBG of 14 days. \(\Delta\Delta\Delta P < 0.001\) vs normal control; \(\Delta P < 0.05, \Delta\Delta P < 0.01, \Delta\Delta\Delta P < 0.001\) vs db/db control

**Fig. 4** Effects of catalpol on random blood glucose in db/db mice. \(\Delta\Delta\Delta P < 0.001\) vs normal control; \(\Delta P < 0.05, \Delta\Delta P < 0.01, \Delta\Delta\Delta P < 0.001\) vs db/db control
Effects of catalpol on OGTT in db/db mice (A), Effect of catalpol on OGTT AUC in db/db mice (B), Effects of catalpol on ITT in db/db mice (C), Effects of catalpol on ITT AUC in db/db mice (D), Effects of catalpol on reducing rate of blood sugar level of ITT in db/db mice (E). △△△ \( P < 0.001 \) vs normal control; * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \) vs db/db control

Effects of catalpol on GSP level

To investigate the long-term hypoglycemic effect of catalpol, glycated serum protein (GSP) level was determined. As shown in Fig. 6, the serum levels of GSP in the db/db mice were significantly higher than that in the normal control group. However, catalpol treatment notably decreased the level of GSP in a dose-dependent manner, compared with the db/db control group. △△△ \( P < 0.001 \) vs normal control; * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \) vs db/db control

Effects of catalpol on globe gene expression in db/db mice

In the present study, we compared gene expression levels in the liver between the db/db control and high-dose (200 mg·kg\(^{-1}\)) or low-dose (100 mg·kg\(^{-1}\)) catalpol-treated mice. There were 47 and 287 genes with significant increase and 117 and 520 genes with significant decrease in expression of more than 2- or 1.5-fold between db/db control and high-dose (200 mg·kg\(^{-1}\)) or low-dose (100 mg·kg\(^{-1}\)) groups, respectively.

As shown in Table 1 and Fig. 7A, of the 287 genes over-expressed in the catalpol-treated mice, the 20 most significant ones included Idh2 [isocitrate dehydrogenase 2 (NADP\(^{+}\), mitochondrial], Cyp46a1 (cytochrome P450, family 46, subfamily a, polypeptide 1), Spon2 (spondin 2, extracellular matrix protein), and Gmeb1 (glucocorticoid modulatory element binding protein 1). The major function of those up-regulation genes were involved in lipid metabolism, response to stress, energy metabolism and cellular processes. Significantly reduced expression genes in the catalpol-treated mice were found for 520 transcripts, such as Gadd45a (growth arrest and DNA-damage-inducible 45 alpha), Tgtp (T-cell specific GTPase), and Gzma (granzyme A). The major function of those down-regulation genes were involved in cell growth and death, immune system, and response to stress. Interestingly,
as shown in Table 3, there were four genes expressed differentially that were involved in glucose metabolism or insulin signaling pathways. Among them, Irs1 (insulin receptor substrate 1), Idh2 (isocitrate dehydrogenase 2 (NADP+), mitochondrial) and G6pd2 (glucose-6-phosphate dehydrogenase 2), which can accelerate glucose metabolism to reduce blood sugar level, were significantly overexpressed in the catalpol-treated mice. The expression of SOCS3 (suppressor of cytokine signaling 3), related to insulin resistance, was significantly reduced.

### Table 1 The top 20 over expression genes in the liver of catalpol-treated db/db mice

<table>
<thead>
<tr>
<th>Probe set</th>
<th>Over expression gene</th>
<th>Function</th>
<th>Signaling pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>1453601_at</td>
<td>Idh2: isocitrate dehydrogenase 2 (NADP+), mitochondrial</td>
<td>Glucose metabolism</td>
<td>TCA</td>
</tr>
<tr>
<td>1417709_at</td>
<td>Cyp46a1: cytochrome P450, family 46, subfamily a, polypeptide 1</td>
<td>Lipid metabolism</td>
<td></td>
</tr>
<tr>
<td>1417860_a_at</td>
<td>Spon2: spondin 2, extracellular matrix protein</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1442601_at</td>
<td>Gmeb1: glucocorticoid modulatory element binding protein 1</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1434087_at</td>
<td>Mthfr: 5, 10-methyltetrahydrofolate reductase</td>
<td>Metabolism of cofactors and vitamins</td>
<td>/</td>
</tr>
<tr>
<td>1422202_at</td>
<td>Thrb: thyroid hormone receptor beta</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1449386_at</td>
<td>Hsd17b6: hydroxysteroid (17-beta) dehydrogenase 6</td>
<td>Lipid metabolism, Metabolism of cofactors and vitamins</td>
<td>/</td>
</tr>
<tr>
<td>1457262_at</td>
<td>Sng1: SMG1 homolog, phosphatidylinositol 3-kinase-related kinase (C. elegans)</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1450188_s_at</td>
<td>Lipg: lipase, endothelial</td>
<td>Lipid metabolism</td>
<td>Glycerolipid metabolism</td>
</tr>
<tr>
<td>1427981_a_at</td>
<td>Csd: cysteine sulfonic acid decarboxylase</td>
<td>Metabolism of other amino acids</td>
<td>Taurine and hypotaurine metabolism</td>
</tr>
<tr>
<td>1425270_at</td>
<td>Kif1b: kinesin family member 1B</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1449065_at</td>
<td>Acot1: acyl-CoA thioesterase 1</td>
<td>Lipid metabolism</td>
<td>Fatty acid elongation</td>
</tr>
<tr>
<td>1437212_at</td>
<td>Zfp420: zinc finger protein 420</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1421262_at</td>
<td>Lipg: lipase, endothelial</td>
<td>Lipid metabolism</td>
<td>Glycerolipid metabolism</td>
</tr>
<tr>
<td>1439795_at</td>
<td>Gpr64: G protein-coupled receptor 64</td>
<td>G protein-coupled receptors</td>
<td>/</td>
</tr>
<tr>
<td>1416021_a_at</td>
<td>Fabp5 // fatty acid binding protein 5, epidermal</td>
<td>Lipid metabolism</td>
<td>Glycerolipid metabolism</td>
</tr>
<tr>
<td>1453565_at</td>
<td>Ndufab1: NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1</td>
<td>Energy metabolism</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td>1417963_at</td>
<td>Pltp: phospholipid transfer protein</td>
<td>Endocrine system</td>
<td>PPAR</td>
</tr>
<tr>
<td>1455182_at</td>
<td>Kif1b: kinesin family member 1B</td>
<td>Cytoskeleton proteins</td>
<td>/</td>
</tr>
<tr>
<td>1430135_at</td>
<td>Dnase2a: deoxyribonuclease II alpha</td>
<td>Cellular Processes</td>
<td>/</td>
</tr>
<tr>
<td>1421102_a_at</td>
<td>Vamp3: vesicle-associated membrane protein 3</td>
<td>Cellular Processes</td>
<td>/</td>
</tr>
</tbody>
</table>

### Table 2 The top 20 down expression genes in the liver of catalpol-treated db/db mice

<table>
<thead>
<tr>
<th>Probe set</th>
<th>Down expression gene</th>
<th>Function</th>
<th>Signaling pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>1449519_at</td>
<td>Gadd45a: growth arrest and DNA-damage-inducible 45 alpha</td>
<td>Cell growth and death</td>
<td>MAPK, FoxO, Cell cycle, p53, Apoptosis</td>
</tr>
<tr>
<td>1449009_at</td>
<td>Tgtp // Tgtp2: T-cell specific GTPase // T-cell specific GTPase 2</td>
<td>Immune system</td>
<td>/</td>
</tr>
<tr>
<td>1417898_a_at</td>
<td>Gzma: granzyme A</td>
<td>Immune system</td>
<td>Neuroactive ligand-receptor interaction</td>
</tr>
<tr>
<td>1419209_at</td>
<td>Cxcl1: chemokine (C-X-C motif) ligand 1</td>
<td>Immune system</td>
<td>Cytokine-cytokine receptor interaction, Chemokine, Toll-like receptor</td>
</tr>
<tr>
<td>1422280_at</td>
<td>Gzmk: granzyme K</td>
<td>Immune system</td>
<td>/</td>
</tr>
<tr>
<td>1451612_at</td>
<td>Mt1: metallothionein 1</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1418722_at</td>
<td>Npg: neutrophilic granule protein</td>
<td>Immune system</td>
<td>/</td>
</tr>
<tr>
<td>1435906_x_at</td>
<td>Gbp2: guanylate binding protein 2</td>
<td>response to stress</td>
<td>/</td>
</tr>
<tr>
<td>1419430_at</td>
<td>Cyp26a1: cytochrome P450, family 26, subfamily a, polypeptide 1</td>
<td>Retinol Metabolic</td>
<td>Metabolic</td>
</tr>
<tr>
<td>1452114_s_at</td>
<td>Igfbp5: insulin-like growth factor binding protein 5</td>
<td>response to stress</td>
<td>/</td>
</tr>
</tbody>
</table>
Table 3  Differentially expressed genes about glucose metabolism and insulin signaling pathway

<table>
<thead>
<tr>
<th>Probe set</th>
<th>Gene</th>
<th>Db/db</th>
<th>High dose</th>
<th>Low dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1423104_at</td>
<td>Irs1: insulin receptor substrate 1</td>
<td>−1.55</td>
<td>1.6</td>
<td>1.77</td>
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<tr>
<td>1453601_at</td>
<td>Idh2: isocitrate dehydrogenase 2 (NADP+), mitochondrial</td>
<td>−4.01</td>
<td>3.3</td>
<td>2.31</td>
</tr>
<tr>
<td>1422327_s_at</td>
<td>G6pd2: glucose-6-phosphate dehydrogenase 2</td>
<td>−1.56</td>
<td>1.88</td>
<td>1.57</td>
</tr>
<tr>
<td>1456212_x_at</td>
<td>Socs3: suppressor of cytokine signaling 3</td>
<td>1.94</td>
<td>−3.52</td>
<td>−2.73</td>
</tr>
</tbody>
</table>

Fig. 7  Differentially expressed genes in the liver of catalpol-treated db/db mice. A: The top 20 over expression genes in the liver of catalpol-treated db/db mice, B: The top 20 down expression genes in the liver of catalpol-treated db/db mice. FC: fold change

Discussion

Type 2 diabetes is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin\(^{16}\). Radix Rehmanniae, the root of *Rehmannia glutinosa*, has hypoglycemic activity in normal and streptozotocin diabetic mice\(^{29}\). The mechanism of the hypoglycemic activity is to
stimulate the secretion of insulin and reduce the glycogen content in the liver of normal mice [12]. Catalpol, the active ingredient of *Rehmannia glutinosa*, is reported to have a significant hypoglycemic effect [11, 13]. To study the hypoglycemic effect of catalpol, we conducted the experiment of the hypoglycemic effect of catalpol in db/db mice. The db/db mouse is a widely studied rodent model of T2DM [17-19]. In our study, the db/db mice developed a stably higher blood sugar level, polydipsia, polyphagia, and insulin resistance, compared with the db/m mice. Furthermore, catalpol treatment also significantly lowered the levels of fasting blood glucose, random blood glucose, and glycosylated serum protein and improved glucose tolerance and insulin resistance via increasing insulin sensitivity, compared with the db/db control. These results implied that catalpol has a significant therapeutic effect on diabetes.

To explore the hypoglycemic action mechanisms of catalpol, we determined the global gene expression in livers of the db/db control, high-dose and low-dose catalpol treated mice using the DNA microarray technology. We found several differential expression genes which may be related with hypoglycemic effect of catalpol, such as SOCS3, Irs1, Idh2, and G6pd2.

Suppressor of cytokine signaling 3 (SOCS3) has emerged as an inhibitor of insulin signaling [21-23]. SOCS expression is tightly regulated at the transcriptional level and is induced by multiple cytokines in different tissues in a cytokine- and tissue-dependent manner [22]. Ruan et al. have found that SOCS3 could be upregulated directly by the induction of increased intracellular ROS in mature 3T3-L1 adipocytes [24]. SOCS3 has been implicated as a mediator by which insulin negatively regulates its own signaling cascade. SOCS3 contributes to both leptin resistance and insulin resistance as a result of increased ceramides synthesis [25-29]. Several studies have shown that removal of the SOCS gene prevents against insulin resistance in obesity [27-28]. As shown in Fig. 8, in insulin signaling pathway, SOCS3 inhibits the phosphorylation of the insulin receptor and down-regulates Irs1 expression via binding to Tyr-960 of the insulin receptor [30-31]. Our results from the present study demonstrated that SOCS expression was up-regulated and Irs1, Idh2 and G6pd2 expression were down-regulated in db/db mice, compared with normal control mice. We also found that expression of T-cell specific GTPase (Tgtp), granzyme A (Gzma), and chemokine (C-X-C motif) ligand 1 (Cxcl1) which are related with immune and inflammation were significantly upregulated. However, catalpol treatment can significantly improve the aforementioned gene expression especially SOCS3.

**Fig. 8 Insulin signaling pathway**

**References**


Researcher SHEN Xiu-Ping has been engaged in research and development of new drugs for 31 years. Her major research focuses on the preclinical pharmacology and toxicology evaluation and research of new drugs. At present, She is also a member of the Applied Pharmacology Committee of the Chinese Pharmaceutical Association, a member of the Chinese Medicine and Natural Medicine Toxicology Committee of the Chinese Toxicology Society.