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ORIGINAL RESEARCH

Venlafaxine Inhibits the Apoptosis of SHSY-5Y Cells Through Active Wnt/β-Catenin Signaling Pathway

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Objective: This study aimed to explore the mechanism of venlafaxine in regulating the apoptosis of SHSY-5Y cells induced by hypoxia.

Methods: The CoCl2-induced neuronal hypoxia model was established based on SHSY-5Y cells. The morphology and related protein expression of SHSY-5Y cells were detected by qPCR, ELISA and Western blot.

Results: Under the condition of hypoxia-induced by CoCl2, the expression of HIF-1α in SHSY-5Y cells was up-regulated and the expression of β-catenin was down-regulated. After adding siRNA targeting HIF-1 α to the culture cell system, down-regulation of β -catenin expression in SHSY-5Y cells was restored. This confirmed the existence of the "hypoxia-HIF -1α-Wnt/β-catenin-depression" axis. Further studies have shown that venlafaxine can alleviate neuronal apoptosis induced by hypoxia by upregulating the Wnt/β-catenin signaling pathway.

Conclusion: Venlafaxine regulates apoptosis induced by hypoxia through the Wnt/β-catenin signaling pathway, which provides a new theoretical basis for the treatment of depression.

Keywords: venlafaxine, Wnt/β-catenin, SHSY-5Y cells, apoptosis

Introduction

Depression is the fourth largest disease in the world, affecting 350 million people worldwide (2015, WHO), with high rates of disability and mortality.[1] Depression causes serious mental problems and cognitive impairment to patients and even threatens their lives, as well as a heavy burden on patients' families and society.[2] It is worth noting that depression usually begins in young people.[3] Therefore, the elaboration of the molecular level of depression and the corresponding early intervention is very important for individuals, families, and even the whole society.[4–9]

Wnt/β–catenin pathway abnormalities are related to the occurrence and development of a variety of diseases (eg, tumors, Alzheimer's disease, metabolic diseases, etc.).[10–15] Down-regulation of the Wnt/β-catenin signaling pathway has been confirmed as an important mechanism of depression. Besides, depression symptoms caused by long-term hypoxia have been widely concerned. Therefore, it is necessary to explore the molecular mechanism between hypoxia and depression.[16] HIF-1α is the most important cytokine expressed by cells in the hypoxic environment,[17,18] which has been shown to inhibit the Wnt/β–catenin signaling pathway in the development of skeletal muscle.

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Venlafaxine is a dual antidepressant for serotonin and norepinephrine reuptake inhibitors.[19–22] Venlafaxine can selectively block the 5-hydroxytryptamine transporter and norepinephrine transporter, and it has a very low affinity for cholinergic, histaminergic, and adrenergic receptors.[23] Its pharmacological properties are unique with no obvious adverse reactions caused by tricyclic antidepressants. Venlafaxine is the first-line treatment drug recommended by disorder prevention guidelines for depressive disorder and general anxiety in China.

SHSY-5Y cells are human bone marrow cells derived from adult human neuroblastomas. Their chromosome number is 47 and the phenotype is Philadelphia chromosome-positive. SHSY-5Y cells are often used in neurological and psychiatric research due to their neuronal characteristics. In this study, SHSY-5Y cells were used to test the hypothesis of "hypoxia-HIF-1α-Wnt/β-catenin-depression" and then to explore the role of venlafaxine in regulating the apoptosis of SHSY-5Y cells caused by hypoxia through the Wnt/β-catenin signaling pathway.

Methods

Cell Culture

We purchased human dopaminergic neuroblastoma cell line, SHSY5Y cells, from the Chinese Type Culture Collection. SHSY-5Y cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin, maintained at 5% CO2 and 37°C. The culture medium was updated twice a week. The experiments were performed using 0.5×106 cells per well under 12-well plates or otherwise specified conditions. In the SHSY-5Y cell culture system, cobalt chloride (CoCl2) was used to create anoxic environment and treated with venlafaxine. The experiments were divided into blank control group, CoCl2 treatment group, venlafaxine and CoCl2 treatment group, and test cells. We detect changes in all aspects of the cell.

siRNA Transfection

According to literature reports, HIF-1α siRNA and β-catenin siRNA were designed using online software. SHSY-5Y cells were transfected at a density of about 60% to 70% as instructed by the Lipofectamine 3000 transfection reagent. The cells were cultured for 48 hours after transfection.[24]

Detection of the Expression of HIF-1A and β-Catenin

The tissues were homogenized with 0.5mL ice-cold lysis buffer. The composition of ice-cold lysate is 20 mM Tris-HCl, 1 mM DTT, 1 mM EDTA, 5 mM MgCl2, 2 mM sodium orthovanadate, 20 μg/mL aprotinin and 1 mM PMSF, pH 7.5. Centrifuge the homogenate at 10,000 x g at 4°C for 20 minutes, and then remove the supernatant. With bovine serum albumin as the standard, the protein concentration was determined using the bicinchoninic acid method. After boiling the sample for 5 minutes (30 μg/mL protein per lane), it was separated by 15% SDS-PAGE and transferred to a nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). Subsequently, the nitrocellulose membranes were blocked with 5% bovine serum albumin for 1.5 h at room temperature, and incubated with anti-PPARα rabbit antibody (1:1000; catalog number ab8934) at 4°C for 12 h. After that, the membrane was washed three times with TBS-Tween 20 buffer and incubated with HRP-conjugated goat anti-rabbit IgG H&L secondary antibody (1:200; catalog number ab6721) for 1 hour at room temperature. GDV for specific frequency bands was measured using Quantity One version 4.62 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Quantitative Real-Time PCR

First-strand cDNA was obtained using the Reverse Transcription System (Promega), Oligo(dT) and Stem-loop Reverse Transcription primer for mRNA and miRNA. Quantitative real-time PCR (qPCR) was performed on LightCycler 480 (Roche, Basel, Switzerland) using SYBR Premier Dimer Eraser™ (TaKaRa, Dalian, China). Data were analyzed using the relative quantitative (244Ct) method.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 4 and SPSS 13.0 software. The results are expressed as mean ± SD. Analysis of variance (ANOVA) was used for the three sets of analysis and the Newman-Keuls test was followed. Student's *t*-test was used to compare the two groups. Kruskal–Wallis and Mann–Whitney *U*-tests were used to analyze the data of non-normal distributions.

Results

Effect of Hypoxia Environment Caused by Cobalt Chloride on SHSY-5Y Cells

In the hypoxia environment induced by CoCl2, the number of apoptotic and necrotic cells was significantly increased (Figure 1A and B) and SHSY-5Y cellular morphology was shrinking (Figure 1C and D). The expression of HIF −1α was up-regulated in the SHSY-5Y cell (Figure 1E). The hypoxic environment induced by cobalt chloride significantly down-regulated the expression of β-catenin (Figure 1F and G) (P<0.05).

The Association of HIF-1α with Wnt/β-Catenin Signaling Pathway

The siRNA targeting HIF-1α was added to the cultured cell system. Cobalt chloride-induced down-regulation of β-catenin expression in SHSY-5Y cells was restored (Figure 2).

Relationship Between Venlafaxine and Apoptosis of SHSY-5Y Cells Induced by Cobalt Chloride

As shown in Figure (Figure 3A–C), venlafaxine can alleviate the apoptosis of SHSY-5Y cells induced by cobalt chloride.

Relationship Between Venlafaxine and Proteins Related to Wnt-β-Catenin Signaling Pathway in SHSY-5Y Cells

Detection of Wnt/β-catenin signaling pathway-associated proteins in apoptotic SHSY-5Y cells induced by CoCl2, which were treated with Venlafaxine. The results (Figure 4) showed that partial Wnt-β-catenin signaling pathway-related proteins' levels of transcribed and translated were significantly increased (P < 0.01).

The Correlation of Venlafaxine and Wnt/β-Catenin Signaling Pathway of Neuronal Apoptosis Induced by Hypoxia

Designing siRNA targeting β-catenin for further experiments. The results showed that venlafaxine could alleviate apoptosis caused by cobalt chloride, but the addition of siRNA targeting β-catenin further aggravated apoptosis (Figure 5).

Discussions

Depression is one of the major refractory diseases in the field of psychosis and has a significant impact on human health.[25] Therefore, it is very important to explore the etiology and molecular mechanism of depression,

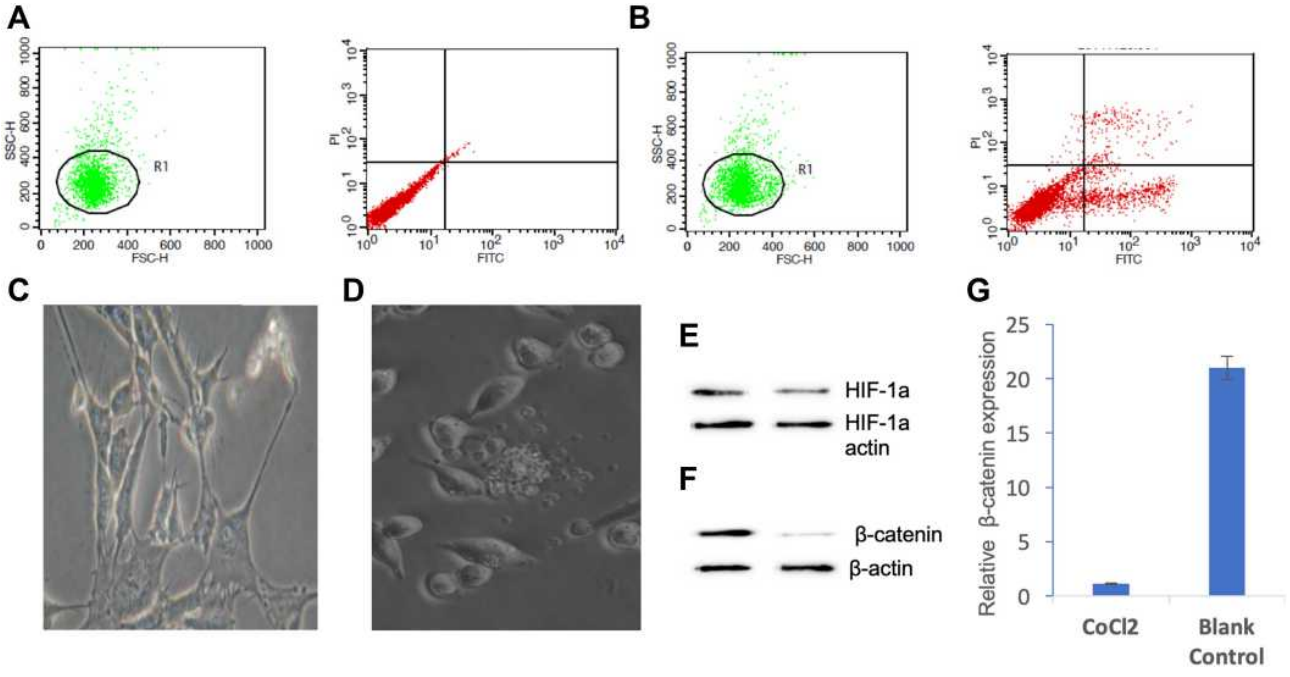

Figure 1 Effect of hypoxic environment caused by cobalt chloride on SHSY-5Y cells. (**A**) The number of apoptotic and necrotic cells in normal culture condition; (**B**) the number of apoptotic and necrotic cells in cobalt chloride-induced hypoxic environment; (**C**) SHSY-5Y cellular morphology in normal culture condition; (**D**) SHSY-5Y cellular morphology in cobalt chloride-induced hypoxic environment; (**E**) the expression of HIF −1α in SHSY-5Y cell; (**F**) cobalt chloride-induced hypoxia inhibited β -catenin expression detected by Western blot; (**G**) the hypoxia-induced by cobalt chloride inhibited the expression of β-catenin at the transcriptional level detected by qPCR (P<0.05).

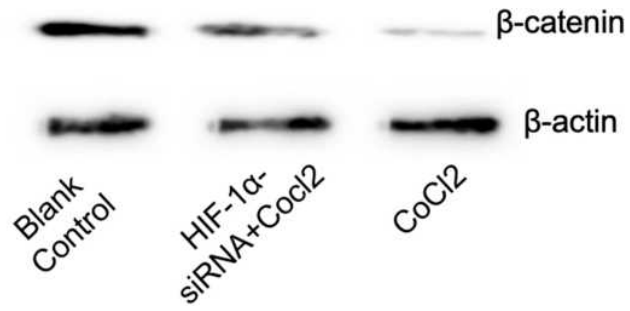

Figure 2 Effect of HIF-1α siRNA on the expression of β-catenin in SHSY-5Y cells induced by cobalt chloride.

including epidemiological etiology and signaling molecular pathways.

The Wnt/β-catenin pathway is an active signaling pathway in the body, which can maintain neuronal and mental homeostasis through BDNF. In the resting state, the phosphorylation of β-catenin is completed by a destructive complex that is dominated by glycogen synthase kinase 3β (GSK-3β), axin, and adenomatous polyposis coli (APC).[26,27] Then it can be recognized by β-TrCP containing the F-BOX domain and specifically binds to the site of phosphorylation of β-catenin, which in turn leads to the ubiquitination of β-catenin. In this way, the latter is eventually degraded by ubiquitin. It should be noted that most of the phosphorylated sites can promote the degradation of β-catenin, while the phosphorylation of certain amino acid sites (such as Ser45 and Thr41) increases the stability of β-catenin. In the Wnt/β-catenin signaling pathway, the Wnt protein firstly binds to the co-receptors formed by the Frizzled (Fz) family receptor and the low-density lipoprotein receptor-associated protein 5 or 6 (LRP5/6), thereby forming ternary protein complex on the surface of cells.

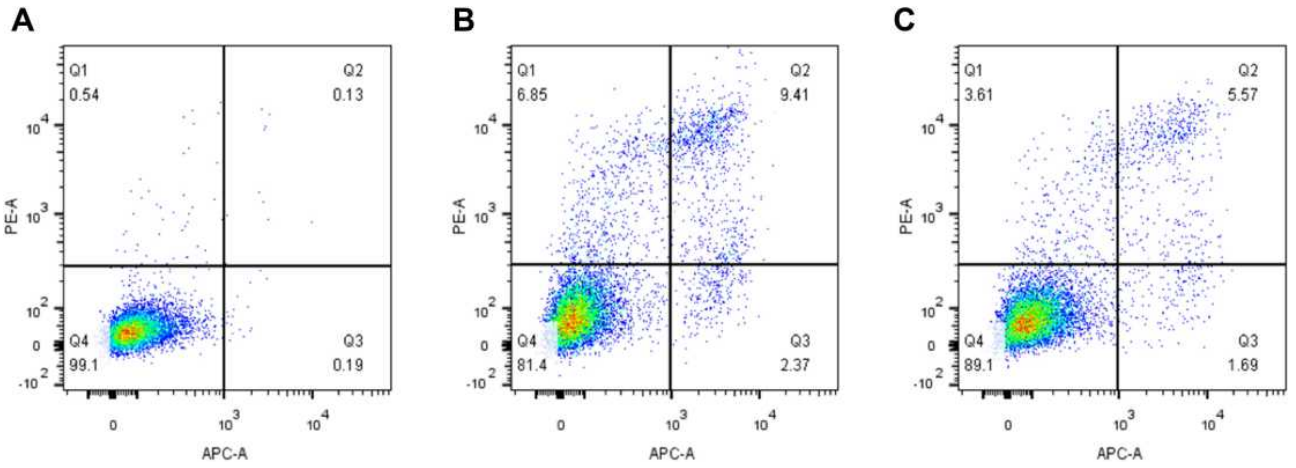

Figure 3 Effect of venlafaxine on apoptosis of SHSY-5Y cells induced by cobalt chloride. (**A**) Control group cells; (**B**) cobalt chloride-induced apoptosis of SHSY-5Y cells; (**C**) venlafaxine alleviated the apoptosis of SHSY-5Y cells induced by cobalt chloride.

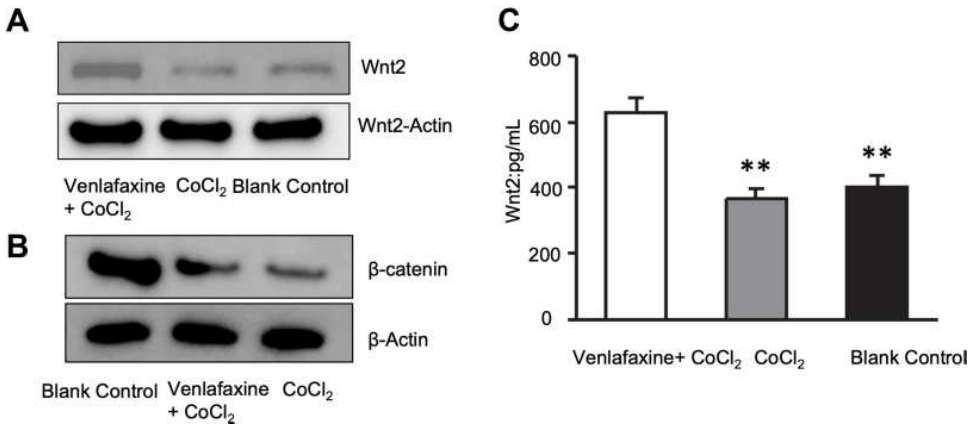

Figure 4 Venlafaxine up-regulated Wnt-β-catenin signaling pathway associated proteins in SHSY-5Y Cell. (**A**) The expression of Wnt2 in three groups; (**B**) the expression of β -catenin in three groups; (**C**) the expression of Wnt2 at the transcriptional level detected by qPCR (**$P < 0.01$).

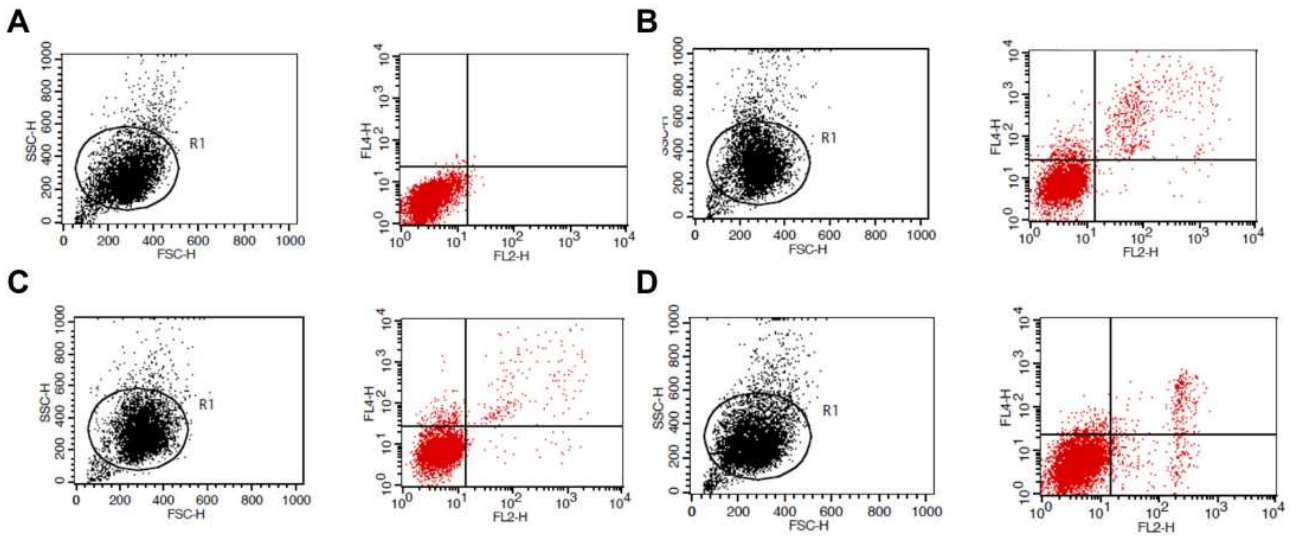

Figure 5 Effect of β-catenin siRNA on apoptosis of SHSY-5Y cells induced by cobalt chloride alleviated by venlafaxine. (**A**) Control group cells; (**B**) cobalt chloride-induced apoptosis of SHSY-5Y cells; (**C**) venlafaxine relieved the apoptosis of SHSY-5Y cells induced by cobalt chloride; (**D**) β-catenin siRNA inhibited the effect of relieved by venlafaxine on apoptosis.

This ternary complex then reacts with intracellular folded protein Dishevell (Dvl) to mediate dissociation of the β-catenin complex (GSK-3[beta], Axin and APC). β-catenin dissociated from GSK-3 β is activated by dephosphorylation and translocated into the nucleus, which mediates the expression of the target gene (such as BDNF) after binding to the LEF1/TCF transcription factor in the nucleus.[28]

Venlafaxine, as a phenylethylamine derivative, is currently the most common antidepressant drug.[29] Various studies have confirmed that venlafaxine can upregulate a variety of Wnt/β-catenin signaling pathway-related proteins and activate the entire Wnt/B–catenin signaling pathway.[30–32] SHSY-5Y cells, a cell line derived from neuroblastoma, can also be infected with various viral vectors while exhibiting neuronal cell characteristics.

In this study, we constructed the model of hypoxia-induced by cobalt chloride on the basis of SHSY-5Y cells. Under the condition of hypoxia-induced by cobalt chloride, we found that the expression of HIF-1α was up-regulated in SHSY-5Y cells and the expression of β-catenin was down-regulated. When siRNA targeting HIF-1α was added to the cultured cell system, the down-regulation of β-catenin expression in SHSY-5Y cells was restored. This confirmed the "hypoxia-HIF-1α-Wnt/β-catenin–depression" axis.[33–36] Further studies have shown that venlafaxine could alleviate neuronal apoptosis induced by hypoxia by upregulating the Wnt/β–catenin signaling pathway.[37–39]

From the results of this study, we can obtain some clinical benefits. Such as Detecting HIF-1α to early diagnose/predict the incidence of depression, finding key nodes in the hypoxia-HIF-1α-Wnt/β-catenin-depressive axis, and screening for effective small molecule targeting agonists or antagonists is expected to effectively treat depression.

Conclusions

In this study, we established models of neuron hypoxia induced by CoCl2 based on SHSY-5Y cells. The results showed that venlafaxine can alleviate neuronal apoptosis induced by hypoxia through upregulating Wnt/β-catenin signaling pathway, which provides a new theoretical basis for the treatment of depression.

Abbreviations

CoCl2, cobalt chloride; ELISA, Enzyme linked immunosorbent assay; qPCR, quantitative real-time PCR; ANOVA, Analysis of variance; GSK-3β, glycogen synthase kinase 3; APC, adenomatous polyposis coli; LRP5/6, lipoprotein receptor-associated protein 5 or 6; DMEM, Dulbecco's Modified Eagle Medium; SD, Standard deviation; BDNF, Brain-derived neurotrophic factor; DTT, Dithiothreitol; HCl, hydrogen chloride; EDTA, Ethylene Diamine Tetraacetic Acid; MgCl2, Magnesium chloride; PMSF, Phenylmethylsulfonyl fluoride; SDS-PAGE, polyacrylamide gel electrophoresis.

Author Contributions

All authors made a significant contribution to the conception, study design, execution, and acquisition of data, analysis and interpretation. They participated in drafting, revising or critically reviewing the article. They gave final approval of the version to be published and agreed upon the journal to which the article has been submitted and agreed to be accountable for all aspects of the work.

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Disclosure

The authors disclose no conflict.

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