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Venlafaxine protects methylglyoxal-induced apoptosis in the cultured human brain microvascular endothelial cells

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HIGHLIGHTS

- Venlafaxine concentration-dependently protects methylglyoxal -induced injury in the cultured HBMEC.
- Venlafaxine protects methylglyoxal-induced apoptosis in the cultured HBMEC.
- Venlafaxine protects methylglyoxal-induced HBMEC injury through PI3K/AKT and JNK pathway.

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ABSTRACT

It was reported that venlafaxine protects microvascular endothelial cells injury in several models. But the mechanisms of venlafaxine protects cell injury still poor understanding. Here, we shows that in the cultured human brain microvascular endothelial cells (HBMEC), we found that venlafaxine protects methylglyoxal (MGO)-induced cell injury, and the venlafaxine significant reduction in the level of reactive oxygen species, down-regulated expression of pro-apoptotic activated caspase-3 and Bax, increased BDNF release and expression of anti-apoptotic Bcl-2 in the cultured HBMEC. Furthermore, we found that venlafaxine inhibits MGO-induced phosphorylation of JNK. Moreover, venlafaxine increased AKT phosphorylation and the protective effects of venlafaxine was inhibited by PI3K/AKT inhibitor. These findings suggest that venlafaxine protects MGO-induced HBMEC injury through PI3K/AKT and JNK pathway as the potential underlying mechanisms of HBMEC injury in diabetes.

1. Introduction

Cerebrovascular disease remains a leading cause of morbidity and mortality in subjects with diabetes distinguished by poor glycemic control and impaired glucose tolerance [21]. It has been documented that hyperglycemia exacerbates ischemic stroke, which is associated with augmented in the size of the infarct and vasodegenerative change [3]. Although many factors contribute to cerebrovascular dysfunction in diabetes, it is now widely accepted that methylglyoxal (MGO) plays a critical role in the progression of diabetic vascular complications [5,12]. In hyperglycemic conditions, levels of precursors of triose phosphate, such as glucose or fructose, are increased. After nonenzymatic fragmentation, high serum levels of MGO were observed in patients with either type 1 or type 2 diabetes [12].

Vascular disorders will induce several biochemical and cellular reactions such as inflammatory response, increased reactive oxygen species (ROS) production, impairment of blood–brain barrier and cell apoptosis [14]. Recent reports have shown that the neuroprotective effects of antidepressant are involved in the improvement of several depression symptoms [8,19]. Venlafaxine is a classical antidepressant and widely used for the treatment of major depression, anxiety, panic disorder, and vasomotor symptoms [7]. Recently, it was reported that venlafaxine protected PC12 cells against corticosterone-induced cell death [16]. These findings provide new insights for venlafaxine as an effective therapeutic agent for diseases with systemic vascular endothelial disorders such as diabetes stroke.

The present study was aimed to demonstrate the protective effect of venlafaxine on MGO-induced injury in the cultured human brain microvascular endothelial cells (HBMEC) and accompanied by identifying the possible mechanism which is responsible for the protection. Data derived from the present study raise the possibility that venlafaxine may be a new strategy to prevent or improve vascular complications associated with diabetes stroke.

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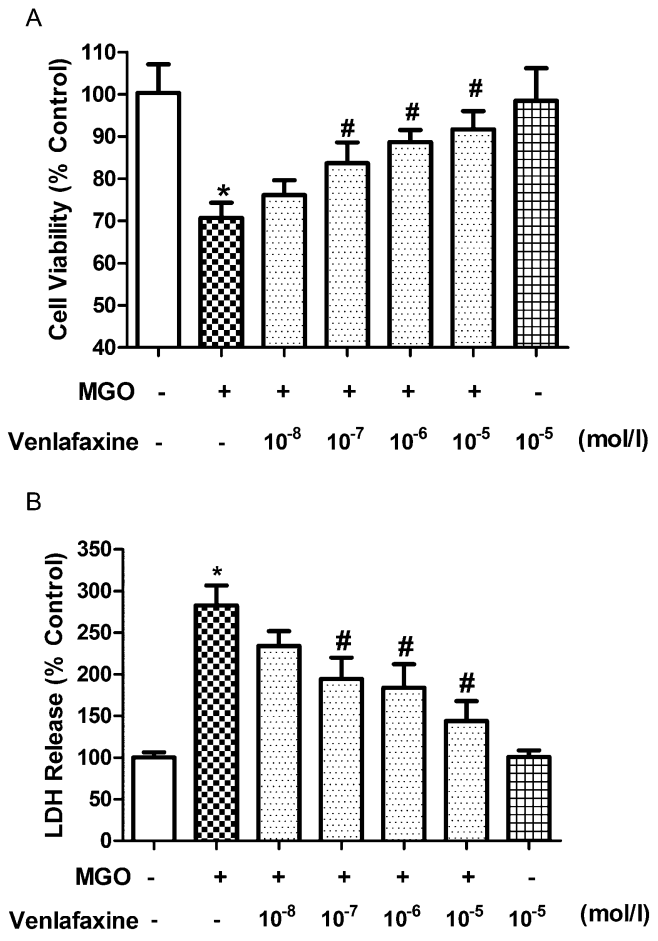

Fig. 1. Venlafaxine protects MGO-induced cell injury in the cultured HBMEC. (A) HBMEC were incubated with venlafaxine (10^{-8}–10^{-5} mol/l), for 20 min before MGO (2 mmol/l) treatment. After 24 h MGO treatment, cell viability was determined by MTT assay. (B) Cell death was assessed by LDH releasing. $^{*}p < 0.05$ versus control group. $^{\#}p < 0.05$ versus MGO group. $n = 4$ repeats.

2. Materials and methods

2.1. Materials

A human brain microvascular endothelial cell line was cultured in RPMI 1640 and characterized for brain endothelial phenotypes as previously described [10]. RPMI 1640, fetal bovine serum, Nu-Serum, MEM non-essential amino acids, sodium pyruvate were purchased from Gibco (Grand Island, USA). LY294002, Akt inhibitor VIII and venlafaxine were purchased from Calbiochem (La Jolla, CA, USA). Methylglyoxal (MGO, 40%, w/v) were purchased from Yuanye Biotech (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). Lactate dehydrogenase (LDH) assay kit and ROS assay kit were purchased from Nanjing Institute of Jianchen Biological Engineering (Nanjing, China). The Caspase-3 assay kit was obtained from Sigma (St. Louis, MO, USA). Mouse anti-β-actin monoclonal antibody was purchased from Abmart (Shanghai, China). Rabbit anti-Bax, anti-Bcl-2, anti-AKT, anti-phospho-AktThr473, anti-JNK and anti-phospho-JNK polyclonal antibody were purchased from Abcam (Cambridge, MA, USA). BDNF enzyme-linked immunosolvent assay (ELISA) kit was purchased from Promega Co. (Madison, WI, USA).

2.2. Cell viability and cell death measurements

HBMEC were treated with 10^{-8}–10^{-5} mol/l venlafaxine 20 min prior to 24 h MGO (2 mmol/l) treatment. Cell viability was determined by the MTT assay [5]. Equal numbers of the cultured HBMEC were exposed to various treatments, and cells were incubated with an MTT solution (50 μl, 5 mg/ml) at 37 °C for 4 h. Next, cells were placed in wells of a microtiter plate and scanned to visualize the color development. Cell survival rates were expressed as percentages of the value of normal cells. Cell death was measured by the amount of LDH released by cells determined by an LDH assay kit according to manufacturer's protocol.

2.3. Measurement of BDNF by ELISA

BDNF concentration in the conditioned media was measured using the EmaxTM Immunoassay System (Promega, Madison, WI, USA) according to the procedures provided by the manufacturer.

2.4. Caspase-3 activity assay

Caspase-3 activity was determined according to the manufacturer's instruction. Briefly, cells were collected and washed with chilled PBS, then suspended in lysis buffer for 15 min on ice. The lysates were centrifuged for 20 min at 10,000 × g, where the supernatants were collected and the protein concentration was calculated. Cell extracts (30 μg) were then incubated in a 96-well microplate with 200 μmol/l acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA) for 2 h at 37 °C. Caspase activity was

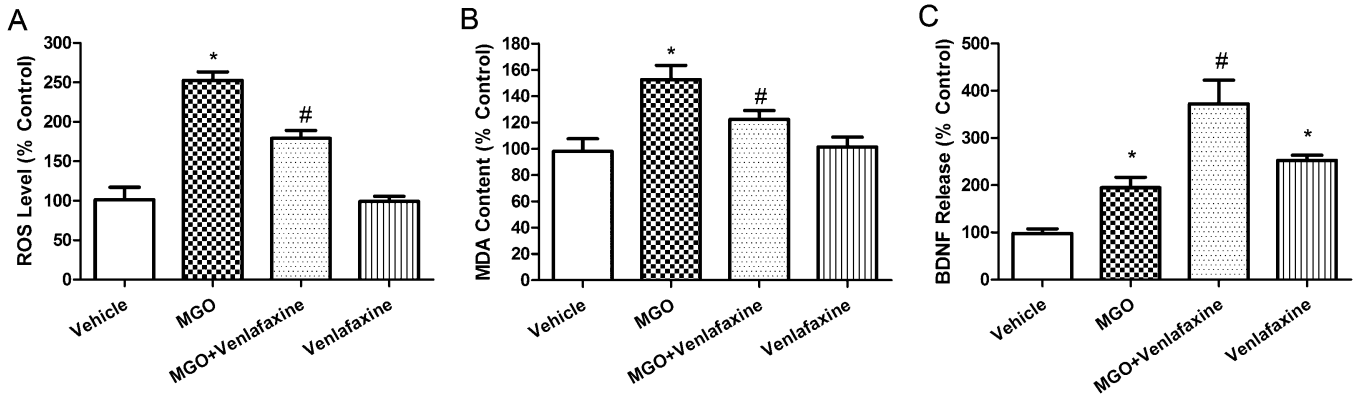

Fig. 2. Venlafaxine protects MGO-induced oxidative damage in the cultured HBMEC. HBMEC were incubated with venlafaxine (10^{-5} mol/l), for 20 min before MGO (2 mmol/l) treatment. After 24 h MGO treatment, analyses of fluorescence intensity values for intracellular ROS level (A), lipid peroxidation manifested by MDA (B) and BDNF release were shown. $^{*}p < 0.05$ versus control group. $^{\#}p < 0.05$ versus MGO group. $n = 4$ repeats.