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Insulin is a potential antioxidant for diabetes-associated cognitive decline via regulating Nrf2 dependent antioxidant enzymes☆

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ABSTRACT

Purpose: To investigate the neuroprotective effects of insulin on diabetic encephalopathy and its mechanism.
Experimental and approach: The diabetic model was established by injection of streptozotocin. Behavior examinations were conducted by the Morris water maze. Histopathological alterations were detected by HE staining. ROS, CAT levels and SOD activity were measured using a microplate reader. *In vitro*, the viability of wild type and knock-down PC12 cells was detected by MTT assay, the morphology of cells was monitored under a microscope. The subcellular distribution of Nrf2 was observed by western blotting and immunohistochemistry.
Key results: Evident oxidative stress injury was observed in diabetic rats and H_2O_2-induced PC12 cells. Insulin not only protect diabetic rat from oxidative stress injury but also significantly inhibited H_2O_2-induced apoptosis and intracellular ROS in cells. In addition, the level of malondialdehyde was reduced, and the activities of superoxide dismutase, catalase and glutathione peroxidase were augmented in both diabetic rats and PC12 cells. Interestingly, insulin promoted the translocation of Nrf2 into the nucleus and activation of downstream antioxidant protein expression. Further, the Nrf2 knockdown cells suffered more serious H_2O_2-induced damage than the wild PC12 cells. Moreover, insulin had no significant protective effect on knockdown cells with H_2O_2-damage.
Conclusion and implications: Collectively, our results suggested that insulin significantly inhibited neuronal damage through the Nrf2 signaling pathway, which regulates endogenous oxidant-antioxidant balance, therefore, insulin may be a potential protective agent for the treatment of oxidative stress-induced diabetic encephalopathy.

1. Introduction

With the aging of the global population, the incidence rates of neurodegenerative diseases such as Alzheimer disease, Huntington disease and diabetic encephalopathy [1–3] have been increased rapidly and such diseases impose a burden on both family and society. Diabetic encephalopathy is a serious complication with cognitive dysfunction of the central nervous system caused by diabetes mellitus. The mechanism of its pathogenesis has not been fully elucidated at present [4], but some researchers observed that it may be related to neuronal cell apoptosis induced by hypoxia or oxygen-free radical injury [5]. Because of dysfunctional metabolism of blood glucose, the basilar membranes of capillaries of the cerebra in most diabetic patients become massive, thereby causing narrowing of the cavity over several years, which in turn causes a decreased blood supply to the brain. Diabetes is more likely with metoprolol than with cavedilol in heart failure [6].

Abbreviations: ARE, Nrf2-antioxidant responsive element; CAT, catalase; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; DCFH-DA, 2',7'-dichlorodihydrofluorescence diacetate; FBS, fetal bovine serum; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; IP-3-K/PKB, phosphatidylinositol-3-kinase/protein kinase B; Keap 1, Kelch-like ECH-associated protein-1; KD, knockdown; Nrf2, NF-E2-related factor 2; MDA, malondialdehyde; MTP, mitochondrial transmembrane potential; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromid; NADPH, triphosphopyridine nucleotide; NC, negative control; PC12 cell, pheochromocytoma cells; Rh123, 2-(6-amino-3-imino-3H-xanthen-9-yl) benzoic acid methyl ester; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin

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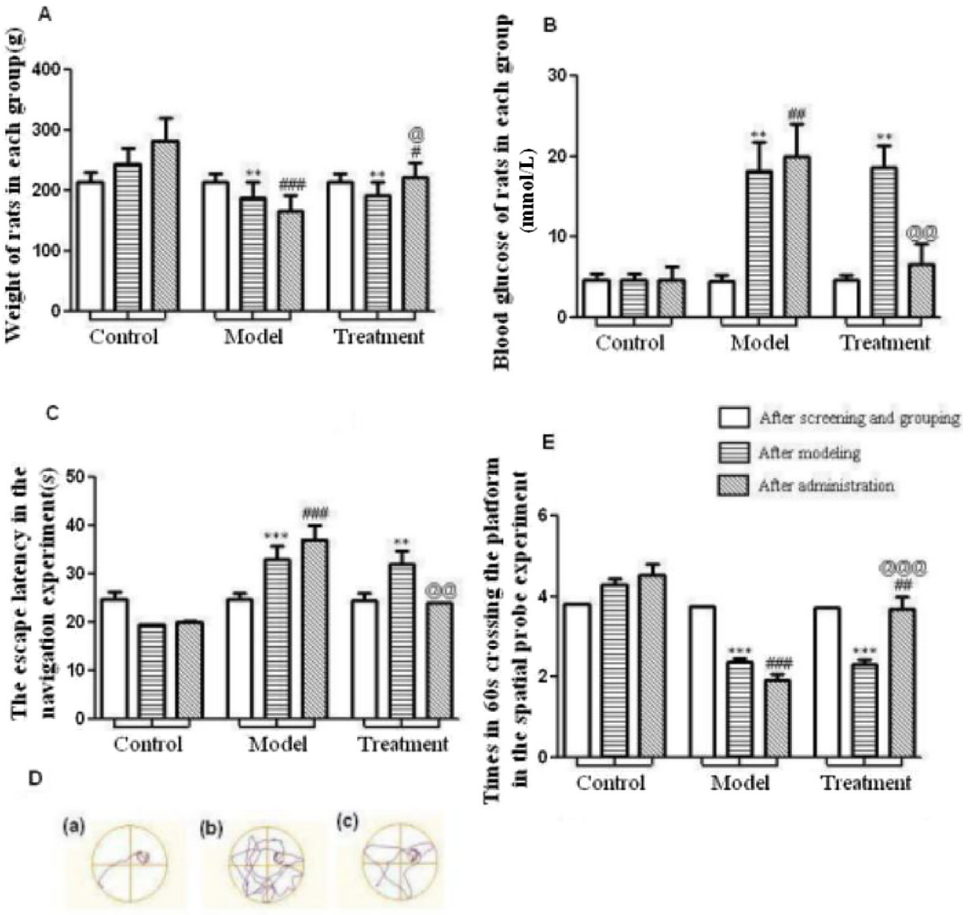

Fig. 1. The effect of insulin on diabetes-associated cognitive decline.
A. The weight of experimental rats.
B. The blood glucose of experimental rats.
C. The escape latency of experimental rats.
D. The orbit of experimental rats in the spatial probe experiment.
E. The time in 60 s crossing the platform in the spatial probe experiment.
In control group, the weight increased with the increase of age, and the blood glucose was in the normal range. In model group, the weight decreased with the increase of age, and the blood glucose was above 13.7 mmol/L. In treatment group, the weight increased significantly with the increase of age, the blood glucose was about 6.68 mmol/L, and it was close to the control group.
For the place navigation test, the escape latency of model group was prolonged, but that of treatment group was shortened after insulin therapy. For the space exploration test, the times that rats in model group passes the platform in 60 s was reduced, but that of treatment group was increased after insulin therapy.
$^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* the control group after model; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, *vs.* the control group after adiministration; $^{@@}p < 0.01$, $^{@@@}p < 0.001$ *vs.* the model after adiministry.

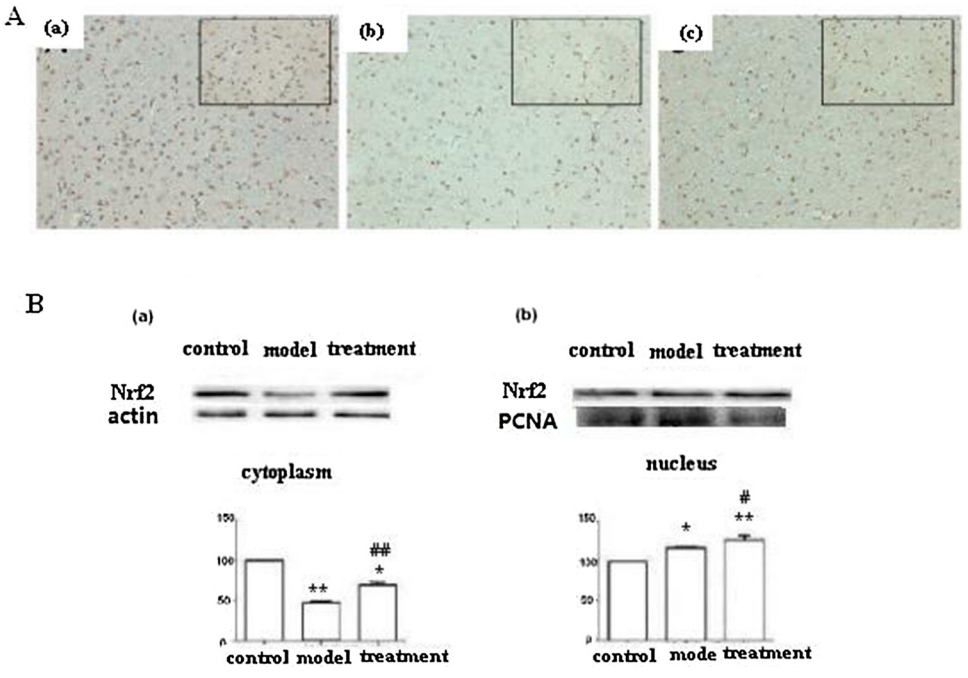

Fig. 2. The expression of Nrf2 on model by immunohistochemistry and western blot.
A. The expression of Nrf2 on diabetic rat by immunohistochemistry.
(a) control, (b) model, (c) insulin treatment.
In control group, the morphology of nerve cells was complete, the space around the cells was dense, the nucleus was clear. In model group, the nerve cells arranged disorderly and sparsely, the cell boundaries were unclear, the cell bodies shrunk, the nuclei condensed. In treatment group, although the arrangement of cells was relatively sparse, the cell bodies of neurons were also shrunk, but the damage had been improved obviously compared with the model group.
Compared with control group, the expression of Nrf2 in model group was increased, but that of treatment group was increased more pronounced with immunohistochemistry.
B. Subcellular distribution of Nrf2 protein in PC12 cells and diabetic rats' brain by western blot.
The cytoplasm expression of Nrf2 on (a) diabetic rat by western blot.
The nucleus expression of Nrf2 on (b) diabetic rat by western blot.
The results of western blot were the same as, and the Nrf2 were transferred from cytoplasm to nucleus. The results of western blot was the same as that of immunohistochemistry, and Nrf2 were transferred from cytoplasm to nucleus.
$^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* model.

However, data demonstrated that the pathway of insulin-receptor signaling is closely related with biological behaviors such as the proliferation and apoptosis of neurons, formation and transmission of synapses, and the energy metabolism of sugar and protein [7,8]. Recent studies suggest that oxidative stress plays a vital role in the pathogenesis of diabetic encephalopathy [4,9].