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The effect of fibroblast growth factor 21 on a cellular model of Alzheimer's disease with emphasis on cell viability and mitochondrial membrane potential

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ABSTRACT

Background and Objective: Alzheimer's disease (AD) is a neurodegenerative disorder which is associated with extracellular accumulation of amyloid beta (Aβ) plaques. AD is accompanied by mitochondrial dysfunction and energy metabolism reduction. Fibroblast growth factor 21 (FGF21) is an endogenous polypeptide which its beneficial effects have been demonstrated on mitochondrial function, energy metabolism regulation and neuroprotection.

Materials and Methods: The present study was performed to investigate the effect of pretreatment with different concentrations of FGF21 [100,200 and 400 nM] on SH-SY5Y cells as a cellular model of AD induced by $A\beta_{(1-42)}$. For induction of cellular model of AD. $A\beta_{(1-42)}$ [20 µM] was added to SH-SY5Y cell medium. Cell viability (MTT assay) and mitochondrial membrane potential changes (Rhodamine 123 fluorescence intensity) were measured using microplate reader.

Results: The results of this study showed that $A\beta_{(1-42)}$ enhances cell damage ($p<0.05$) and decreases mitochondrial membrane potential ($p<0.05$). Pretreatment of SH-SY5Y cells with FGF21 increased cell viability ($p<0.05$- 0.001) and mitochondrial membrane potential ($p<0.05$-0.01) in a concentration-dependent manner.

Conclusion: Taken together, the results of this study suggest that FGF21 prevents cell death induced by $A\beta_{(1-42)}$ in SH-SY5Y cells. It seems that the beneficial effects of FGF21 are mediated through mitochondrial membrane potential maintenance.

1. Introduction

Alzheimer's disease (AD) is a progressive form of dementia that impair memory and learning in older people (1). Neurofibrillary tangles composed from hyperphosphorylated tau protein and senile plaques composed of amyloid-β (Aβ) peptide at cortical and subcortical areas of brain are two important hallmarks for AD (2). According to recent studies, there is a correlation between diabetes and neurodegeneration in AD (3). Increasing resistance to insulin causes neuronal loss around senile plaques (4). Imperfect glucose metabolism is related with learning and memory impairment (5) that leads to the progressive dementia (6). Some studies show that glycemic control can reduce the cognition impairment (7), but others do not confirm these results (8). The chief functions of FGF21 are the control of glucose and lipid metabolism with increasing adipose tissue glucose uptake and lipolysis (9).

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Many intracellular stressors including mitochondrial disturbances, inadequacy of autophagy, perturbation of Ca^{+2} homeostasis in endoplasmic reticulum (ER) can augment FGF21 production (10). Recently, it has been found that FGF21 has a main role in controlling oxidative stress in humans (11).

Studies have shown that oxidative stress-induced ER stress stimulates promotor of FGF21 gene through activation of activating transcription factor 4 (ATF4) (12). Oxidative stress is correlated with a great number of metabolic diseases including type 2 diabetes mellitus, obesity and resistance to insulin (13). Administration of FGF21 systemically decreases the level of plasma glucose, lipid insulin and glucagon. It also get better sensitivity to insulin, energy consuming, and obesity in animal models of insulin resistance (14, 15). Recently, FGF21 is known as a forceful regulator of insulin-independent metabolism (16). The activity of FGF21 depends on its binding to fibroblast growth factor receptor (tyrosine kinase receptor) and β-klotho (single-pass transmembrane protein) as a co-receptor (17). Binding of FGF21 to its receptor and co-receptor leads to fibroblast growth factor receptor substrate 2 (FRS2) phosphorylation. Phosphorylated FRS2 stimulates other components such as RAS-RAF-mitogen-activation protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)-serine-threonine protein kinase (AKT), the activator of transcription (STAT) and phosphoinositide phospholipase C (PLC) γ (18). Recently, expression of FGF21 has been revealed in different regions of brain particularly the area containing dopaminergic neurons including striatum, substantia nigra, hippocampus and cortex (19). In addition, FGF21 plays a major role in providing of glucose homeostasis through interaction with liver and brain (20). On the other hand, FGF21 has an important effect on behavior and cognition so that the high degree of FGF21 expression in mice changes its behavior in dark and light phase. It seems that FGF21 has an effective role in controlling of circadian rhythm in the brain (21). Here, we investigated FGF21 effect on cell viability and mitochondrial membrane potential(MMP) in a cell model of AD in SH-SY5Y neuroblastoma cells.

2. Materials and Methods

Human SH-SY5Y neuroblastoma cells (Pasteur Institute, Iran) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM glutamine, 100U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum (FBS) in a humidified atmosphere at 37°C with 5% CO2. For formation of cellular model of AD, the wells containing model group were treated with 20 μM of $A\beta_{(1-42)}$ for 24 h.

2.1. Cell viability

The cell viability was determined using methyl thiazol tetrazolium bromide (MTT) assay. SH-SY5Y neuroblastoma cells were seeded in a 96-well microtiter plate at a density of 5000 cells/well at a volume of 200 microliter. Groups for evaluation were as follows: 3 Wells containing SH-SY5Y neuroblastoma cells with no treatment (control) for 48 h, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 100 nM treatment, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 200 nM treatment, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 400 nM treatment, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h $A\beta_{(1-42)}$ 20 μM, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h 20 μM $A\beta_{(1-42)}$ with 24 h FGF21 100 nM pretreatment, 3 wells containing SH-SY5Y neuroblastoma cells with 24 h 20 μM $A\beta_{(1-42)}$ with 24 h FGF21 200 nM pretreatment, and 3 wells containing SH-SY5Y neuroblastoma cells with 24 h 20 μM $A\beta_{(1-42)}$ with 24 h FGF21 400 nM pretreatment.

2.2. Mitochondrial membrane potential (MMP)

SH-SY5Y neuroblastoma cells were seeded in a 48-well microtiter plate at a density of 20000-25000 cells/well at a volume of 1 ml and groups were as follows: 5 wells containing SH-SY5Y neuroblastoma cells with no treatment (control) for 48 h, 5 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 100 nM treatment, 5 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 200 nM treatment, 5 wells containing SH-SY5Y neuroblastoma cells with 24 h FGF21 400 nM treatment, 5 wells containing SH-SY5Y neuroblastoma cells with 24 h $A\beta_{(1-42)}$ 20 μM, 5 wells containing SH-SY5Y neuroblastoma cells

with 24 h 20 μM Aβ $_{(1-42)}$ with 24 h FGF21 100 nM pretreatment, 5 wells containing SH-SY5Y neuroblastoma cells with 24 h 20 μM Aβ $_{(1-42)}$ with 24 h FGF21 200 nM pretreatment, and 5 wells containing SH-SY5Y neuroblastoma cells with 24 h 20 μM Aβ $_{(1-42)}$ with 24 h FGF21 400 nM pretreatment.

2.3. Statistical analysis

All data are presented as means ± SEM and were statistically analyzed by parametric one way ANOVA followed by Tukey *post hoc* test. Differences were significant if *p* value was less than 0.05.

3. Results

3.1. Effect of FGF21 on the viability of Aβ $_{(1-42)}$-treated human SH-SY5Y neuroblastoma cells

Application of FGF21 to human SH-SY5Y neuroblastoma cells showed no significant effect on the cell viability. Treatment of SH-SY5Y neuroblastoma cells with Aβ caused a considerable decrease in cell viability ($p<0.05$). Different concentrations of FGF21 significantly increased the viability of SH-SY5Yneuroblastoma cells ($p<0.05$-0.001; Fig.1).

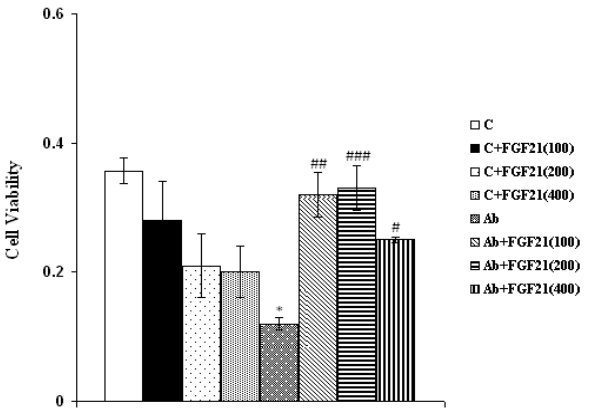

Fig. 1. Effect of FGF21 on viability of amyloid beta-exposed human SH-SY5Y neuroblastoma cells. SH-SY5Y cells were pretreated with 100, 200, and 400 nM of FGF21 for 24 h and then exposed to amyloid beta (20 μM) for 24 h. MTT assay was used to determine cell viability. C means control. Values are means ± SEM. * P<0.05 (Versus Control); # P<0.05, ## P<0.01, ### P<0.001(Versus Ab).

3.2. Effect of FGF21 on the mitochondrial membrane potential of human SH-SY5Yneuroblastoma cells

Treatment of human SH-SY5Y neuroblastoma cells with FGF21 caused no change in mitochondrial membrane potential (MMP) of human SH-SY5Yneuroblastoma cells, but Aβ significantly reduced MMP ($p<0.05$). Application of FGF21 to Aβ-treated human SH-SY5Y neuroblastoma cells enhanced MMP in a dose-dependent manner ($p<0.05$-0.01; Fig. 2).

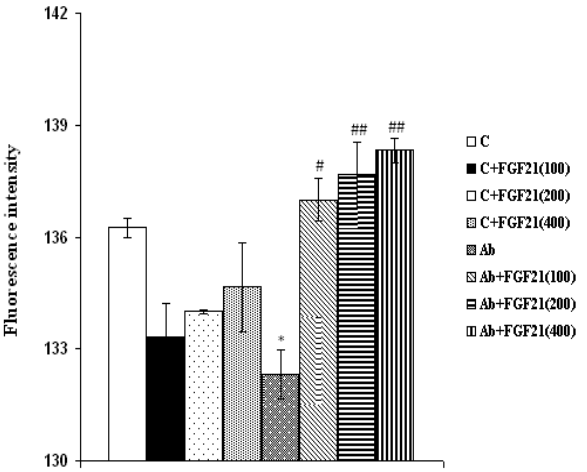

Fig. 2. Effect of FGF21 on amyloid beta-induced rhodamine density alteration as an indicator of mitochondrial membrane potential in human SH-SY5Y neuroblastoma cells. SH-SY5Y cells were pretreated with 100, 200, and 400 nM of FGF21 for 24 h and then exposed to amyloid beta (20 μM) for 24 h. C means control. Values are means ± SEM. * P<0.05(Versus Control); # P<0.05, ## P<0.01 (Versus Ab

4. Discussion

According to obtained results in the present study, it was demonstrated that treatment of human SH-SY5Y neuroblastoma cells with FGF21 in three different concentrations does not has not any effect on cell viability and MMP. However, in Aβ-treated human SH-SY5Y neuroblastoma cells, cell viability and MMP significantly decreased.

Consistent with our study, it has been demonstrated that ROS production is induced by Aβ in cultured cells, subcellular fractions and animal models of diseases (22-24). In neurons, Aβ with activation of NADPH oxidase increases ROS production. Moreover, Aβ intensifies oxygen radicals synthesis by mitochondria (25, 26). In another mechanism, the fibrillar or soluble form of Aβ with activation of microglia causes the production of ROS (27, 28). In abnormal conditions, because the amount of produced ROS is much more than the neuronal antioxidant content and activity, it impairs the neurons. In addition, since mitochondria is the most sensitive organelles to ROS, impaired mitochondria may cause to apoptosis (29). ROS with depolarization of mitochondrial membrane potential decreases $\Delta\psi_m$. Normal $\Delta\psi_m$ is essential for cell survival and accurate function of mitochondria for ATP synthesis (30). Depolarization of mitochondrial membrane potential that is associated with early stages of neuronal apoptosis (31), decreases the Rh123 (a lipophilic cation) accumulation by mitochondria (32). As it has been shown in our study, Aβ-induced ROS production could decrease $\Delta\psi_m$ and neuroblastoma viability.

In the present study, it was also revealed that FGF21 treatment of Aβ-treated human SH-SY5Y neuroblastoma cells improves their viability and mitochondrial membrane potential. FGF21, as a growth factor, belongs to FGF19 family that its major function is regulation of peripheral cell metabolism (33).

Recently, it was revealed that mutations in the genome of mitochondria increase FGF21 expression (34). Some diseases are accompanied by mitochondrial mutations and resulting elevation of FGF21 (35). The relation between diabetic complications and the plasma level of FGF21 indicate that FGF21 has a critical role in reducing of lipotoxicity and programmed cell death (36). It seems that FGF21 is a factor that responds to stress or cell injury and has a mediator role in curative effects of drugs that are used for metabolic diseases treatment (37).

Since FGF21 can pass the blood brain barrier and enter brain tissues (38, 39), it seems that FGF21 has a main role in regulation of brain metabolism. Thus, treatment of dopaminergic neurons with FGF21 increase mitochondrial respiratory capacity (40). Also, FGF21 exerts neuroprotective effects and causes better cognition through restriction of tau-phosphorylation (41). Some studies indicate that insufficiency in autophagy and disturbance in mitochondrial function cause to increase the expression of FGF21. Also, FGF-21 inhibits glutamate-evoked excitotoxicity and cell death in aging cerebellar granular cells through increasing p-Akt-1Ser473 (42). The beneficial effects of FGF21 on cell viability and mitochondrial membrane potential in our study may be attributed to the above-mentioned mechanisms. It is obvious that much more studies are necessary to unravel detailed mechanisms of FGF21 functions.

Collectively, the results of this study suggest that FGF21 prevents cell death induced by $A\beta_{(1-42)}$ in SH-SY5Y cells. It seems that the beneficial effects of FGF21 are mediated through mitochondrial membrane potential maintenance.

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