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Neuroprotective effect of nicorandil in 6-OHDA induced in vitro model of parkinson's disease

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Abstract

The purpose of this study was to examine the consequences of opening KATP channels using nicorandil in an in vitro 6-OHDA Parkinson's disease model conducted in SH-SY5Y cells. To establish Parkinson's disease model in vitro, 200µM 6-OHDA administered to the cells for 24h. Half an hour before the 6-OHDA administration, SH-SY5Y cells were pre-treated with nicorandil (10, 100, 500 and 1000µM). After 1 day, cell viability was determined with the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and lactate dehydrogenase (LDH) assays. Oxidative stress was assessed with superoxide dismutase (SOD), glutathione (GSH), reactive oxygen species (ROS) and malondialdehyde (MDA) analyses. We found that 6-OHDA increased LDH leakage, and cellular apoptosis in SH-SY5Y cells. 6-OHDA aggravated oxidative stress by increasing ROS and MDA and eventually promoted apoptosis by increasing mRNA expression levels of Caspase-3 in SH-SY5Y cells, while pretreatment with nicorandil attenuated these toxic effects of 6-OHDA by suppressing oxidative stress and apoptosis. Considering its neuroprotective role in addition to its effects on oxidative stress and apoptosis, nicorandil may be a useful agent in the treatment of Parkinson's disease.

Keywords: Nicorandil, KATP, 6-OHDA, parkinson's disease, SH-SY5Y

Introduction

Parkinson's disease (PD) is one of the most common neurological diseases with a progressive nature, which is more common in old age. Affecting around 1% to 2% of adults older than 65 and 4% of adults older than 80, PD is estimated to reach more than 12 million patients worldwide by approximately 2050 [1]. Dopaminergic neuron loss in the substantia nigra is one of the main pathophysiological features of PD, and this loss leads to impaired motor performance [2]. The clinical characteristics in PD patients are tremor, resting bradykinesia, rigidness, and postural instability. Despite the fact that, specific etiology and pathogenesis of neuronal degeneration are not well understood, many mechanisms have been considered to be related with PD pathogenesis including abnormal protein aggregation, reactive oxygen species (ROS) accumulation which results in oxidative stress and cell death in dopaminergic neurons [3,4].

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6-hydroxydopamine (6-OHDA) is a neurotoxicant that has been extensively utilized to induce in vivo and in vitro PD model. Oxidative stress and apoptosis come into play in cell toxicity in the 6-OHDA-induced PD model. In addition, it was reported that it produces ROS and causes energy depletion with mitochondrial dysfunction [5].

Interestingly, ATP-sensitive potassium channels (KATP), which are involved in many molecular pathways including cell survival and protect neurons against neurotoxin-induced apoptosis, are of great interest. KATP-channels are extensively found in especially midbrain. Although the precise role of KATP-channels in neurodegenerative diseases is controversial, evidence from previous studies has suggested that activation of KATP-channels may protect neuronal cells against toxic insults from neurotoxins [6]. Activation of KATP-channels has been well documented to have neuroprotective and anti-inflammatory properties against neurotoxin-induced PD [7]. Nicorandil is a KATP-channel opener that has been revealed to preserve mitochondrial membrane potential in neurons with antiapoptotic, antioxidant and anti-inflammatory properties and exerts neuroprotective effects in brain [6].

Thus, the purpose of this study was to examine the consequences

of opening KATP channels using nicorandil in an in vitro 6-OHDA PD model conducted in SH-SY5Y cells in the context of oxidative stress and apoptosis.

Materials and Methods

Cell culture

SH-SY5Y cells were received from Pharmacology Department at Ataturk University (Turkey). SH-SY5Y cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) accompanied with 10% fetal bovine serum (FBS), antibiotic solution. Cells were incubated at 37 °C in a 5% CO_2. SH-SY5Y cells were seeded at the density of 5000 per well in 96 well-plates. To establish PD model in vitro, 200 µM 6-OHDA administered to the cells for 24 h. Half an hour before the 6-OHDA administration, SH-SY5Y cells were pre-treated with nicorandil (10, 100, 500 and 1000 µM) [8].

MTT assay

3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the cell viability. 20µl MTT solution (SigmaAldrich) was given to all wells and waited for 4h at 37ºC. Supernatants were then replaced with 150µm DMSO and the optical density was determined by spectrophotometer at 490nm.

LDH release assay

Lactate dehydrogenase (LDH) is an intracellular enzyme found in almost all cells. LDH is released from cells when membrane integrity is disrupted. To evaluate the cytotoxicity, LDH activity were assigned by colorimetric method using an LDH assay kit (LDH Assay kit, Elabscience, United States) in accordance with kit procedure. Optical density was assessed at 450nm.

Oxidative stress parameters

Superoxide dismutase (SOD), Glutathione (GSH), malondialdehyde (MDA) and ROS were determined using a commercial kit (Elabscience, United States) according to manufacturer instructions as described before [9]. Levels of ROS were measured using ELISA kit (LSBio, United states) according to manufacturer's guide. Optical density was assessed at the wavelength of 450nm.

Molecular analysis

mRNA extraction and cDNA synthesis were carried out as previously described with RNeasy easy kit (Qiagen, Hilden, Germany) Real-time RT-PCR analysis also were performed as previously described [10]. Briefly, total RNA isolation was conducted with RNeasy Mini Kit. The obtained RNA was stored at–80°C. The RNA samples were converted into cDNA using High-Capacity cDNA Reverse Transcription Kit. The obtained cDNA was measured by nanodrop spectrophotometry (EPOCH Take 3 Plate, BioTek), and obtained cDNA was stored at -20 °C. Relative mRNA caspase-3 expression levels were determined by Rotor-Gene Q (QIAGEN). β-actin was utilized as the reference gene. The target gene expression levels were compared with the housekeeping gene β-actin. The sequences of specific primers were as follows: Caspase-3:forward,5′-TTTTCAGTCCGGGGACAAAC-3′, reverse,5′-GGGCAGCCGAGAATAACAAT-3′,β-actin: forward,5′-CAAGGTGGGTGTCTTTCCTG-3′,reverse, 5′-GATCCACACGGAGTACTTGC-3′. The obtained results were analyzed as fold changes using the $2^{-\Delta\Delta Ct}$ method.

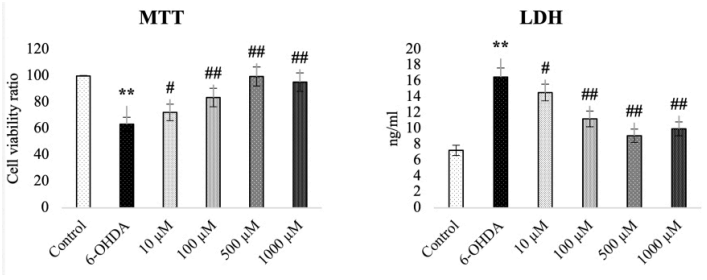
Statistical analyses

All analyses were performed by one-way analysis of variance (ANOVA) with post hoc Tukey's test (IBM SPSS 22.0) ($p<0.05$). Data were presented as mean ± SD.

Results

MTT and LDH assay

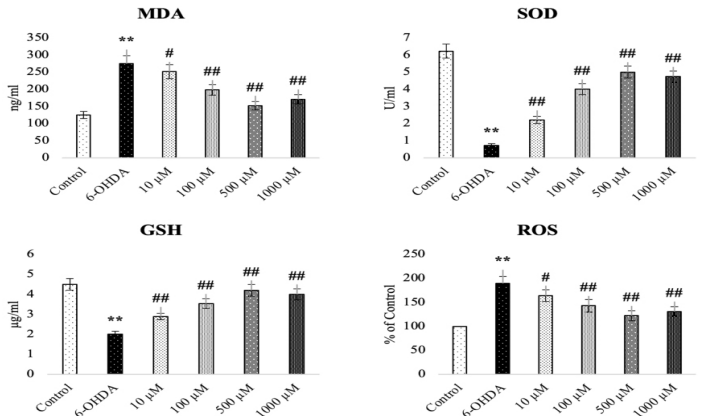
The 200µM 6-OHDA caused a significant decrease in viable cell ratio in the MTT assay and elevated LDH release in LDH assay. Our results demonstrated that after 24h nicorandil pretreatment at especially 100, 500 and 1000µM concentrations nicorandil elevated the cell proliferation and our results evidenced that nicorandil markedly increased viable cell ratio. Additionally, nicorandil significantly decreased the levels of LDH in comparison to 6-OHDA group (Fig. 1).



Figur 1. Effects of nicorandil on the cell viability (MTT and LDH) of SH-SY5Y cells. Data are expressed as the means ± SD. ** p<0,001 vs control group, # p<0,05 vs 6-OHDA group, ## p<0,001 vs 6-OHDA group.

Oxidative stress results

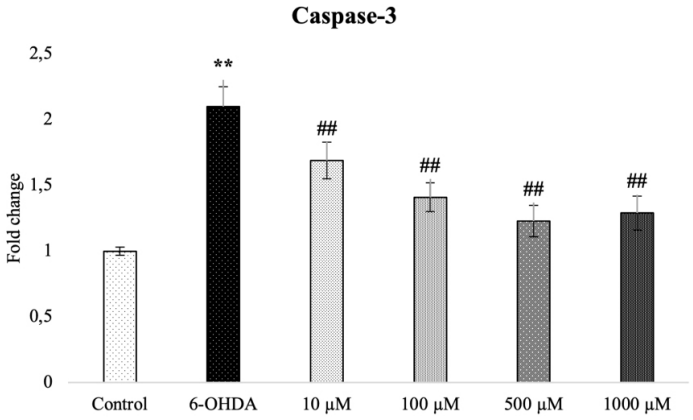
SOD and GSH activity were significantly decreased in 6-OHDA group while MDA and ROS levels significantly increased in comparison to control group. Activities of SOD and GSH were significantly increased in nicorandil groups, whereas MDA and ROS concentrations of those groups were significantly lower than 6-OHDA group (Fig. 2).



Figur 2. Effects of nicorandil on the oxidative stress parameters (MDA, SOD, GSH and ROS) of SH-SY5Y cells. Data are expressed as the means ± SD. ** p<0,001 vs control group, #p<0,05 vs 6-OHDA group, ##p<0,001 vs 6-OHDA group

mRNA expressions of Caspase-3

The mRNA expression of Caspase-3 was significantly up-regulated in the 6-OHDA group. Nicorandil administration significantly decreased the expression of caspase-3 in comparison to 6-OHDA group. (Fig. 3).



Figur 3. Effects of nicorandil on the apoptosis of SH-SY5Y cells. Data are expressed as the means ± SD. ** p<0,001 vs control group, ## p<0,001 vs 6-OHDA group

Discussion

In this study, the neuroprotective effects of KATP channel opener nicorandil against 6-OHDA neurotoxicity in SH-SY5Y cells was evaluated for the first time.

In this study, we observed that SH-SY5Y cell viability was significantly reduced in the 6-OHDA group. However, administration of nicorandil especially at the dose of 500µM increased cell viability. In addition to the cell viability assay results, apoptosis was found to be decreased in a dose-dependent manner in all nicorandil groups. Various studies have shown that nicorandil has beneficial effects in neurodegenerative diseases both in vitro and in vivo [11-14]. In addition, in a previous report, it was shown that opening KATP channels with diazoxide ameliorated the symptoms in a PD model in rats [15]. Nicorandil may be effective in neurodegenerative diseases such as PD, as it acts by opening KATP channels like diazoxide. It has been shown by Teshima et al. that nicorandil can prevent apoptosis by protecting mitochondrial metabolism, activating KATP channels and stopping cell death in neurons damaged by oxidative stress [6].

Oxidative damage is accepted as a key pathogenic process involved in neurodegenerative diseases [16]. Excess generation of ROS may modulate neuronal cell function and cause neurodegenerative diseases, such as PD [17,18]. Neurons are particularly sensitive to the oxidation of polyunsaturated fatty acids due to their high lipid content in their cell membranes. When oxidation of polyunsaturated fatty acids is increased, cell membranes are damaged and transmission of nerve impulses are disrupted [19,20]. Lipid peroxidation products are potentially used as biomarkers of cellular oxidative damage. Thus, inhibiting ROS generation is a valuable strategy to downregulate oxidative stress. Nicorandil has been reported as a strong antioxidant agent in neurodegenerative disease [21]. Like previous reports, we observed that nicorandil declined the ROS and MDA concentrations and elevated the activity of SOD and GSH. Robin et al. have reported that, nicorandil was effective in preventing cell apoptosis in neurons and in cerebral ischemia via its antioxidant properties [7].

Induction of apoptosis with 6-OHDA is one of the crucial factors in the pathogenesis of in vitro PD model. In addition, it is known that apoptosis of dopaminergic neurons plays an important role in movement disorders and mortality in PD patients. [22]. Caspase-3 is an important member of the cysteine protease family concerned with in the mitochondrial apoptotic pathway [23]. Nicorandil-treatment has been previously demonstrated to increase neuron cell density, decrease cells death in a rat model of stroke by Owjfard et al [24]. Current study showed that nicorandil has antiapoptotic effects by downregulating caspase-3 expression. All these results together imply that nicorandil prevents cell death and protects cells from neurotoxins.

Conclusion

These findings indicated that nicorandil, with its antioxidant and antiapoptotic features might be a potential candidate for neuroprotection in PD. In conclusion, current study demonstrated that nicorandil alleviates 6-OHDA-induced neurotoxicity in SH-SY5Y cells. Moreover, with current study it was clarified that the neuroprotective effects of nicorandil were related with inhibition of oxidative stress and prevention of apoptosis. Thus, our results emphasize the effects of nicorandil for prevention and treatment of PD. Considering its neuroprotective role in addition to its effects on oxidative stress and apoptosis, nicorandil may be a useful agent in the treatment of PD.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
N/A (Since the SH-SY5Y cell line was used in our study, it does not require ethics committee approval.)

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