ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2023.23014

Neuroprotective Effects of Pregnenolone Against Oxidative Damage in a 6-Hydroxydopamine-Induced Parkinson's Disease Cell Model Using SH-SY5Y Cell Line

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ABSTRACT

Objective: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons, leading to motor impairments and cognitive deficits. Despite advances in understanding PD's pathophysiology, effective therapeutic interventions are limited. Oxidative stress, resulting from the overproduction of reactive oxygen and nitrogen species, plays a crucial role in PD progression. Neurosteroids, naturally occurring brain steroids, have been implicated in dopamine signaling regulation and are disrupted in PD. Pregnenolone (Prgn), a neurosteroid, has shown potential neuroprotective properties and antioxidant effects.

Methods: In this study, we investigated the neuroprotective potential of Prgn in an in vitro PD model using SH-SY5Y cells treated with 6-hydroxydopamine (6-OHDA). Pregnenolone pretreatment significantly improved cell viability and reduced oxidative stress induced by 6-OHDA exposure. Moreover, Prgn treatment led to the modulation of antioxidative biomarkers, restoring cellular redox balance.

Results: Our findings suggest that Prgn exerts neuroprotective effects against 6-OHDA-induced neurotoxicity in SH-SY5Y cells by reducing oxidative stress and enhancing cell survival. These results support the exploration of Prgn as a potential therapeutic agent for mitigating PD progression.

Conclusion: In conclusion, our study demonstrates that Prgn, a neurosteroid, exhibits promising neuroprotective effects in an in vitro model of PD by attenuating 6-OHDA-induced oxidative damage and preserving the viability and functional integrity of SH-SY5Y cells.

Keywords: 6-OHDA, in vitro, oxidative stress, Parkinson's disease, pregnenolone

INTRODUCTION

Parkinson's disease (PD) is a gradually worsening neurodegenerative condition marked by the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor difficulties and cognitive impairments.[1-3] Despite significant advances in understanding the pathophysiology of PD, the available therapeutic interventions remain limited and often fail to halt or reverse the relentless progression of the disease.[4] An essential characteristic of PD is the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which leads to oxidative stress. The accumulation of ROS and RNS triggers a cascade of detrimental events, including lipid peroxidation, protein misfolding, and DNA damage, culminating in neuronal dysfunction and death. Consequently, there is a growing interest in exploring novel neuroprotective agents that can effectively mitigate oxidative damage and ameliorate the course of PD.[5]

Various research studies have found that neurosteroids, which are naturally occurring steroids produced and active in the brain, can influence the communication of dopamine within a brain region called the striatum.[6] It is important to note that these natural substances are disrupted or imbalanced in the brains, cerebrospinal fluid, and blood of individuals with PD and in animal models of PD.[7]

Pregnenolone (Prgn), a neurosteroid synthesized in the brain, has attracted considerable attention due to its potential neuroprotective properties.[8] Pregnenolone has been involved in several cellular

Received: 04.07.2023
Accepted: 28.08.2023
Publication Date: 14.09.2023

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Cite this article as: Ferah Okkay I, Yeşilyurt F, Okkay U. Neuroprotective effects of pregnenolone against oxidative damage in a 6-OHDA-induced Parkinson's disease cell model using SH-SY5Y cell line. *Pharmata* 2023;3(4):74-77.

processes, such as neurogenesis, synaptic plasticity, and anti-inflammatory mechanisms.[9] Moreover, several studies have suggested that Prgn exerts antioxidant effects by scavenging free radicals and modulating antioxidant enzyme activities, thereby reducing oxidative stress-induced cellular injury.[10] To examine the neuroprotective potential of Prgn in a PD context, we employed a well-established in vitro model of PD using the SH-SY5Y cell line treated with 6-OHDA. This model mimics the key features of PD, enabling us to assess the efficacy of Prgn in attenuating 6-OHDA-induced oxidative damage and its subsequent neurotoxic effects. In this study, we aimed to elucidate the impact of Prgn on the viability and functional integrity of SH-SY5Y cells following 6-OHDA exposure. Despite numerous studies investigating the effects of neurosteroids in PD, this study represents the first investigation of Prgn's antioxidative effects on SH-SY5Y cells in an in vitro setting. The findings of this study hold significant implications for developing novel therapeutic strategies that target oxidative stress and its deleterious consequences in PD.

METHODS

Cell Culture

We used a type of human neuroblastoma cells called SH-SY5Y (ATCC Cat. CRL-2266) for our cellular model. These cells were grown in a special liquid called Dulbecco's Modified Eagle Medium (DMEM), mixed with 10% fetal bovine serum and 1% penicillin/streptomycin solution. SH-SY5Y cells are commonly used in studies related to PD because they share many characteristics with dopaminergic neurons.[4] Moreover, growing and differentiating SH-SY5Y cells are costeffective. Since these cells are derived from humans, they express specific proteins and variations not naturally present in primary cultures from rodents.[4,11] After letting the cells adhere for 24 hours, we exposed them to a substance called 6-OHDA at a concentration of 200 µM for 24 hours to induce stress. Prior to this stress, we pre-treated the cells with different concentrations (25 µM, 50 µM, and 100 µM) of Prgn for half an hour. After 24 hours, we measured cell viability.[4]

Cell Viability Test

To measure cell viability, we used a test called the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. We added MTT solution (Sigma-Aldrich, MO, USA) to each well, following the instructions provided in the kit. After a specific incubation period, we dissolved the formazan precipitate in 150 µL of a chemical called Dimethyl sulfoxide (DMSO). Then, we used a spectrophotometer (BioTek Instruments, Winooski, USA) to read the absorbance values at 540 nm.[4] This allowed us to assess the viability of the cells.

Measuring Oxidative Stress Markers

To evaluate oxidative stress levels, we used the enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Texas, USA) as per the kit's instructions.[12] These kits allowed us to measure the levels of 2 important markers: TAC (total antioxidant capacity) and TOS (total oxidant status). The measurement was performed by reading the absorbance of the samples at 450 nm using a spectrophotometer.[13]

Statistical Analyses

For data analysis, we utilized a statistical method called 1-way analysis of variance along with post hoc Tukey's test using IBM Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) version 22.0 software.[14,15] In this study, we considered P values less than .05 ($P < .05$) as statistically significant, indicating that the observed results were unlikely due to chance. The data are presented as mean ± SD, which allowed us to show the average value for each group along with the variation or spread of the data around the mean.

RESULTS

Cell Viability

We observed that when the SH-SY5Y cells were exposed to 6-OHDA, their cell viability significantly decreased, indicating a harmful effect of 6-OHDA on the cells (Figure 1). The pre-treatment with Prgn noticeably increased cell viability compared to the group exposed to 6-OHDA alone, suggesting that Prgn has the ability to promote cell proliferation under the influence of 6-OHDA ($P < .05$). This result indicates that Prgn could potentially counteract the damaging effects of 6-OHDA and promote the survival and growth of SH-SY5Y cells.

Oxidative Stress Results

Considering that the production of harmful oxygen radicals and products of lipid peroxidation are associated with the disease

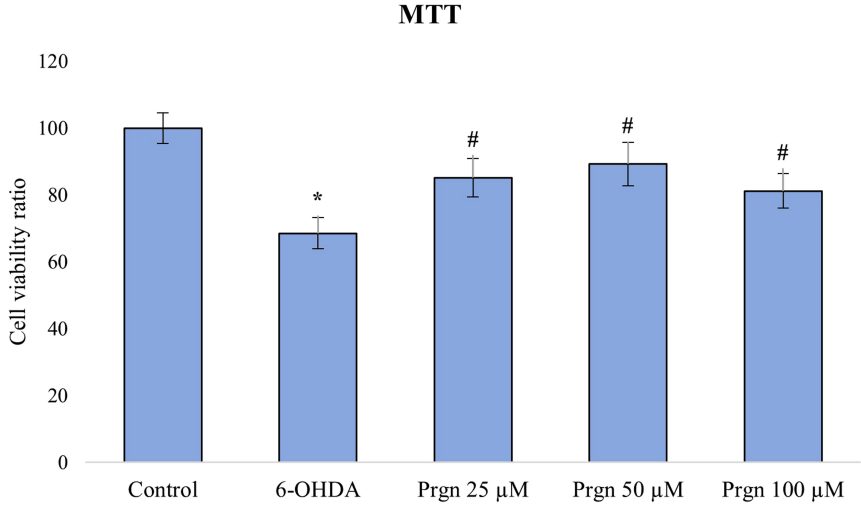

Figure 1. Effects of pregnenolone on the cell viability ratio.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

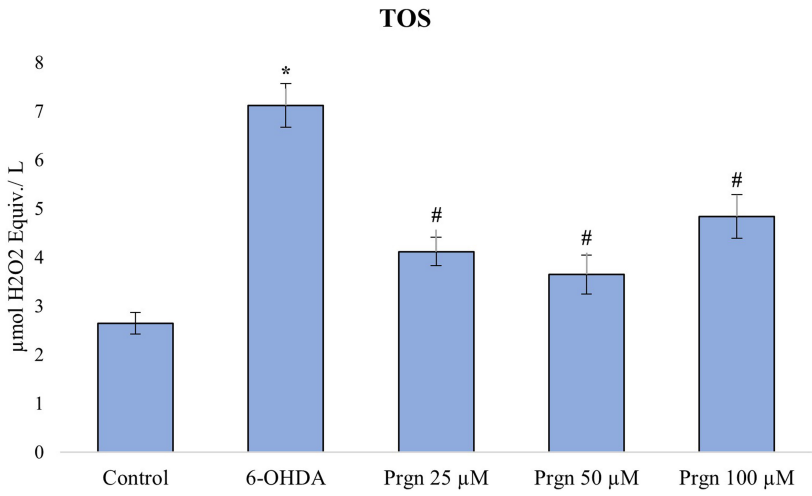

Figure 2. Effects of pregnenolone on the total oxidant status levels.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone; TOS, total oxidant status. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

process of PD, we investigated specific biomarkers related to oxidative stress and antioxidant defense. When the SH-SY5Y cells were exposed to 200 µM 6-OHDA, the levels of TOS markedly increased in comparison with the control group ($P < .05$) (Figure 2). This indicated that 6-OHDA triggered oxidative stress in the cells. Additionally, 6-OHDA caused a significant reduction in the activity of TAC within the SH-SY5Y cells. Interestingly, when we pre-treated the cells with Prgn before exposing them to 6-OHDA, the oxidative burden induced by 6-OHDA was remarkably alleviated ($P < .05$), as depicted in Figure 3. This suggests that Prgn has the potential to counteract the oxidative damage caused by 6-OHDA, thereby protecting the cells from oxidative stress. Moreover, Prgn treatment increased the activity of TAC in the SH-SY5Y cells exposed to 6-OHDA ($P < .05$), indicating an enhancement of the cellular antioxidant defense. Taken together, these results imply that Prgn might play a beneficial role in mitigating the harmful effects of oxidative stress in PD.

DISCUSSION

Parkinson's disease is a disabling neurodegenerative condition characterized by the gradual degeneration of dopaminergic neurons in the substantia nigra, resulting in motor deficits and cognitive dysfunction.[16] Pregnenolone, a neurosteroid synthesized in the brain, has been the subject of research regarding its potential relationship with PD. Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra region of the brain, leading to motor dysfunction.[7] Recent studies have explored the role of Prgn in modulating neuroinflammatory responses, oxidative stress, and neuronal survival mechanisms.[10] It is proposed that Prgn might exert neuroprotective effects by influencing various pathways implicated in PD pathology. However, the exact mechanisms through which Prgn could impact PD progression remain a topic of ongoing investigation.

The quest for effective neuroprotective strategies to ameliorate PD pathology remains a significant challenge in current research. In this study, we utilized a well-established in vitro PD model, utilizing the SH-SY5Y cell line exposed to 6-OHDA, to investigate the potential neuroprotective effects of Prgn. The choice of SH-SY5Y cells as a cellular model is grounded in their relevance and widely accepted use in PD studies, as they exhibit numerous features characteristic of dopaminergic neurons and present a cost-effective approach for investigation.[4,17-19]

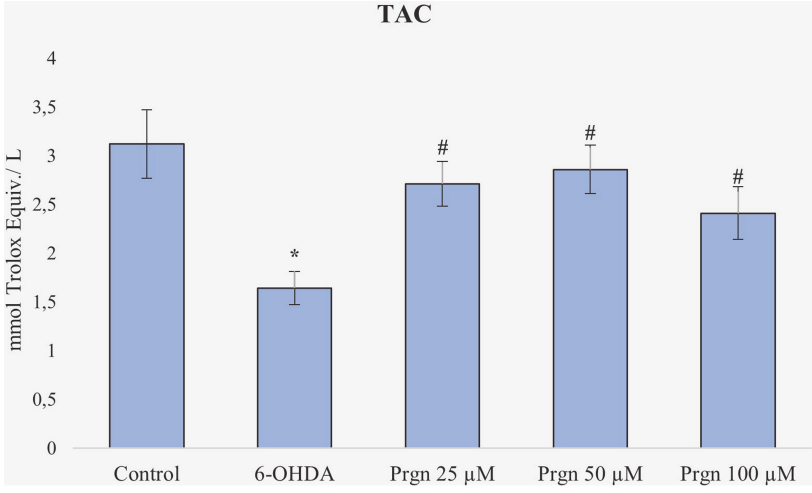

Figure 3. Effects of pregnenolone on the total antioxidant capacity levels.
Data are expressed as the means ± SD. 6-OHDA, 6-hydroxydopamine; Prgn, pregnenolone; TAC, total antioxidant capacity. *$P < .05$ vs. control group, #$P < .05$ vs. 6-hydroxydopamine group.

Importantly, the human origin of the differentiated SH-SY5Y cell culture allowed us to investigate human-specific proteins and isoforms that are not naturally present in rodent primary cultures, enhancing the translational relevance of our findings.[20] The MTT assay, an established method to assess cell viability, was employed to evaluate the impact of Prgn on cellular survival following 6-OHDA-induced stress. Our results unequivocally demonstrated that Prgn pre-treatment significantly increased cell viability in the presence of 6-OHDA, signifying a protective effect against 6-OHDA-induced cytotoxicity. This observation suggests that Prgn's administration may promote cell proliferation and counteract the deleterious consequences of 6-OHDA, potentially exerting a beneficial influence in mitigating the neurodegenerative processes associated with PD. Oxidative stress is a crucial contributor to PD pathogenesis, characterized by an imbalance between ROS production and the cellular antioxidant defense mechanisms.[21] To elucidate the mechanism underlying Prgn's protective effects, we examined oxidative stress-related biomarkers, including TAC and TOS. Exposure to 6-OHDA led to a marked increase in TOS levels, indicative of heightened oxidative stress within the SH-SY5Y cells. In contrast, pre-treatment with Prgn effectively attenuated the 6-OHDA-induced oxidative burden, restoring cellular redox balance and enhancing the activity of TAC. These results demonstrate that Prgn exerts a significant antioxidative effect, conferring cellular protection against oxidative damage in the context of our PD model. The collective findings of this study provide compelling evidence for the neuroprotective potential of Prgn in an in vitro PD model induced by 6-OHDA. By positively influencing cell viability and mitigating oxidative stress, Prgn emerges as a promising candidate for further investigation as a therapeutic intervention in PD. The identification of Prgn's beneficial effects on crucial cellular processes implicated in PD pathology opens new avenues for the development of innovative treatment strategies aimed at attenuating the relentless progression of this devastating neurodegenerative disorder. Nevertheless, the translation of these findings to in vivo and clinical studies is warranted to corroborate the full therapeutic potential of Prgn and its underlying mechanisms of action. Overall, our study provides valuable insights into the field of neuroprotection and brings us closer to the prospect of developing novel therapeutic interventions for PD.

Ethics Committee Approval: Ethical approval was not required as this study was conducted using a commercially acquired cell line.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – U.O., I.F.O.; Design – U.O., I.F.O.; Supervision – U.O., I.F.O.; Resources – I.F.O., F.Y., U.O.; Materials – I.F.O., U.O.; Data Collection and/or Processing – I.F.O., F.Y., U.O.; Analysis and/or Interpretation – U.O., F.Y., I.F.O.; Literature Search – I.F.O., U.O.; Writing Manuscript – U.O., I.F.O.; Critical Review – I.F.O.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

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