Research Article / Araştırma Makalesi

Protective Effects of Resveratrol Carbon Dots Against 6-OHDA-Induced Neurotoxicity in SH-SY5Y Cells

Resveratrol Karbon Noktalarının SH-SY5Y İnsan Nöroblastoma Hücrelerinde 6-OHDA Kaynaklı Nöronal Hücre Ölümü Üzerinde Koruyucu Etkileri

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Abstract

Background: We aimed to investigate the ability of resveratrol carbon dots (RES C-Dots) to protect SH-SY5Y cells from oxido-inflammatory stress and apoptosis caused by 6-hydroxydopamine (6-OHDA).
Materials and Methods: In vitro PD model was generated in SH-SY5Y cells by administering of 200 µM 6-OHDA for 24 hours. Different concentrations of RES C-Dots (12.5, 25, and 50 µg/mL) were applied to the cells 30 minutes before administration of 6-OHDA.
Results: We observed that application of RES C-Dots prevented cell death induced by 6-OHDA and maintained cell viability. As expected, RES C-Dots prevented oxidative damage induced by 6-OHDA - by strengthening the total amount of antioxidants and lowering the total amount of oxidants in SH-SY5Y cells. Similarly, RES C-Dots markedly alleviated the secretion of inflammatory factors (TNF-α and IL-1β) promoted by 6-OHDA. Furthermore, RES C-Dots prevented apoptosis induced by 6-OHDA by suppressing caspase-3 mRNA expression level.
Conclusions: RES C-Dots rescued SH-SY5Y cells from 6-OHDA- induced damage by modulating the oxido-inflammatory and apoptotic response. This report indicates enounces that RES- synthesised C-Dots may have promising curative potential for PD.

Key Words: Parkinson Disease, 6-OHDA, RES C-Dots, SH-SY5Y cells

Öz

Amaç: Bu çalışmada Resveratrol karbon noktalarının (RES-KN) 6-OHDA'nın SH-SY5Y insan nöroblastoma hücrelerininde neden olduğu oksidoinflamatuar stres ve apoptozdan koruma potansiyelini araştırmayı amaçladık.
Materyal ve Metod: SH-SY5Y hücreleri, in vitro PH modelini indüklemek için 24 saat boyunca 200 µM 6-OHDA'ya maruz bırakıldı. Hücrelere, 6-OHDA uygulamasından 30 dakika önce farklı konsantrasyonlarda RES KN (12.5, 25, and 50 µg/mL) uygulandı.
Bulgular: Özellikle, RES KN uygulaması sonucu 6-OHDA'nın neden olduğu hücre ölümü etkili bir şekilde engellendiğini ve SH-SY5Y hücrelerinde hücre canlılığı önemli ölçüde korunduğunu gözlemledik. RES-KN, SH-SY5Y hücrelerinde toplam antioksidanları güçlendirerek ve toplam oksidanları düşürerek 6-OHDA kaynaklı oksidatif hasarı önledi. Benzer şekilde, RES-KN, 6-OHDA kaynaklı inflamatuvar faktörlerin (TNF-a ve IL-1β) salınımını önemli ölçüde azalttı. Ayrıca RES-KN, kaspaz-3 mRNA ekspresyonunu baskılayarak 6-OHDA'nın neden olduğu apoptozu engelledi.
Sonuç: RES-KN'lar, oksido-inflamatuar ve apoptotik yanıtı modüle ederek SH-SY5Y hücrelerini 6-OHDA kaynaklı nörotoksisiteden kurtardı. Bu çalışma, RES'den sentezlenen KN'lerin PH tedavisinde umut verici terapötik potansiyele sahip olabileceğini düşündürmektedir.

Anahtar Kelimeler: 6-OHDA, RES-KN, parkinson hastalığı, SH-SY5Y hücreleri

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Introduction

Increases in reactive oxygen species (ROS) can lead to protein oxidation, DNA damage, alteration of ion channels, and disruption of mitochondrial membrane potential, ultimately resulting in neuronal cell death (1). The brain is particularly vulnerable to oxidative stress because it consumes large amounts of oxygen, contains high levels of fatty acids that are susceptible to peroxidation, and has a weak antioxidant capacity (2). The development of ROS has been associated with neurodegenerative diseases such as Parkinson's disease (PD) (2, 3).

PD is one of the most common brain disorders that occur with age. Levodopa and/or dopamine agonists, used as the first choice in the therapy of PD, show their symptomatic effect (4). On the other hand, antioxidant therapy, although a hopeful option based on oxidative stress and inflammation in Parkinson's disease, has largely failed to meet initial expectations (2). The potential application of flavonoids, plant-derived agents characterized by their antioxidant properties, and their combination with nanomedicine approaches could advance highly effective therapeutic strategies for the treatment of PD (5).

Resveratrol (RES) is a natural polyphenol found in grapes, peanuts, rhubarb, and many other plants (5). It has been stated that RES protects neurons from oxidative damage and toxicity and prevents apoptotic neuronal death (PD) (6, 7). Because of its poor water solubility, unstable chemical structure, short biological half-life, degradation by isomerization upon pH changes, rapid metabolism, and clearance (8), it has low bioavailability. Since successful treatment of PD relies on a high degree of bioavailability of the drug, new strategies are needed. These difficulties can be overcome by encapsulating the drug in a nanomaterial that can be made from a variety of materials (9).

Here, a new antioxidant strategy for the treatment of PD was investigated via carbon dots (C dots) synthesized using RES. C-dots are very tiny nanoscale carbonaceous particles (<10 nm) presenting outstanding physicochemical features, including small size with large specific surface area, excellent drug loading and release, prolonged drug half-life, quality blood brain barrier infiltration and suitable protection against enzymatic degradation e.t.c (9, 10). Bioactive residues on C-dots' surfaces provide them with greater biological activity in comparison to the molecular precursors (10). It was informed, for example, that C-dots synthesized from aspirin exhibited greater anti-inflammatory features relative to the individual molecule aspirin (11).

6-hydroxydopamine (6-OHDA), a potent neurotoxin that damages dopaminergic neurons (DANs), is commonly employed to stimulate in vivo and in vitro experimental PD models. 6-OHDA generates intracellular ROS as well as other free radicals and prevents mitochondria to activate apoptosis cascades (12). The SH-SY5Y cells demonstrate many features of substantia nigra neurons and are so appropriate for utilization as an in vitro model to research the death of DANs (13). Therefore, this study aimed to develop a PD model using SH-SY5Y cells.

As far as we know, the influence of RES C-dots on 6-OHDA-induced SH-SY5Y neurotoxicity has not been explored thus far. We hypothesized that RES C-dots might prevent neuronal cells from 6-OHDA-provoked oxidative damage by acting as both powerful antioxidant and antiapoptotic inducers. Also, we here report for the first time the potentiality of RES C-dots to prevent apoptotic death of 6-OHDA treated SH-SY5Y cells by reducing lipid peroxidation, strengthening antioxidant capacity, as well as by ameliorating neuronal inflammation.

Materials and Methods

Cell Cultures

The Homosapien Bone Marrow Neuroblastoma (SH-SY5Y) cell line was taken from Biology Department at Erzincan Binali Yildirim University (Erzincan, Turkey). 10% Fetal Bovine Serum (FBS) containing antibiotic solution was added to the cells in Dulbecco's Modified Eagle's Medium (DMEM).and kept at 37°C with 5% CO_2. To develop the PD model in SH-SY5Y cells, 200 µM 6-OHDA (Merck, Germany) was applied to each well for 24 h as in our previous study (14). The cells were pretreated with 50 µM pure RES (Merck, Germany), and various dosages (12.5, 25, and 50 µg/mL) of RES C-Dots for half an hour before 6-OHDA-exposure. RES C-Dots, which were prepared with pure RES qua the single carbonaceous precursor via hydrothermal synthesis and had approximately 3nm diameters, were kindly donated by Assoc. Prof. Dr. Kemal Volkan Özdokur (Erzincan Binali Yildirim University, Erzincan, Turkey).

MTT assay

The cell viability was calculated by the 3-[4,5 dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) analysis. The MTT reagent (5 mg/ml; 20 µl; Sigma-Aldrich) was appended to all wells for 4h and then the medium was exchanged with 150 µm DMSO. Then, absorbance was assigned at 570 nm with a microplate reader (Multiskan GO, USA) (14).

Biochemical analysis

TAC and TOS levels were measured to estimate oxidative damage-induced. TAC and TOS concentrations were appraised at an emission of 660 nm and 530 nm, respectively with commercial kits (Rel assay Diagnostics, Turkey). Besides oxidative stress parameters, amounts of inflammatory markers including tumor necrosis factor (TNF)-α, and interleukin (IL)-1β in cells were detected with ELISA kits (Elabscience, USA), accompanied by the manufacturer's guidelines. The corresponding absorbance was specified at 450nm.

Molecular Analysis

Extraction of total mRNA and converting RNA to cDNA were performed with the RNeasy and cDNA synthesis kits, respectively (Thermo Scientific, USA) as in our previous study [14]. The level of caspase-3 relative mRNA expression was determined with Rotor-Gene 6000 (Corbett Life Science, Australia).

β-actin was utilized as the standard gene. Caspase-3 expression was compared with the β-actin gene using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

All data were assessed with a one-way analysis of variance with the Tukey post hoc test (IBM SPSS 22.0) and $p < 0.05$ accepted meaningful. Findings are mean ± SD.

Table 1. The sequence of the primers

Genes	Primer Sequence (5′–3′)
Caspase-3	Forward;5′-TTTTCAGTCCGGGGACAAAC3′
	Reverse;5′-GGGCAGCCGAGAATAACAAT-3′
β-actin	Forward; 5′-CAAGGTGGGTGTCTTTCCTG-3′
	Reverse; 5′-GATCCACACGGAGTACTTGC-3′.

Results

RES C-Dots Prevents 6-OHDA-Evoked Toxicity in SH-SY5Y Cells

Analysis of cell viability was carried out to evaluate whether the RES C-dots display considerable protective performance towards 6-OHDA causing neuronal damage. Figure 1. depicts the effect of the RES C-dots on cell viability in SH-SY5Y cells upon 6-OHDA exposure. As expected, 200 µM 6-OHDA exposure markedly affected the viability of SH-SY5Y cells, in which viability was observed up to 51.4% (p<0,001). Conversely, the pre-treatment of RES C-Dots markedly elevated the SH-SY5Y cells viability applied with 6-OHDA to the level of 66.9% (p<0,05), 73.5% (p<0,001), and 84.4% (p<0,001) at 12.5, 25, and 50 µg/mL concentrations, respectively.

RES C-Dots Alleviates 6–OHDA Promoted Oxidative Damage in SH-SY5Y Cells

The TAC levels were markedly lower ($p < 0.001$) in the 6-OHDA group than in the control cells while the TOS value was importantly elevated ($p < 0.001$) in comparison with the control cells. TAC levels in RES C-Dots at 12.5, 25, and 50 µg/mL concentrations were remarkably higher than the 6-OHDA group ($p < 0.001$), whereas the TOS value of those groups was outstandingly lower than the 6-OHDA group.

RES C-Dots Diminished 6-OHDA-Related Inflammation in SH-SY5Y Cells

As shown in Figure 3, the highest TNF-α and IL-1β levels were observed in the 6-OHDA group ($p < 0.001$). Same time the increase of TNF-α and IL-1β levels caused by 6-OHDA decreased in all concentrations of RES C-Dots treatment groups. The reduction in the TNF-α and IL-1β levels in the 12.5, 25, and 50 µg/mL RES C-Dots groups were statistically significant compared 6-OHDA group ($p < 0.05$, $p < 0.001$, and $p < 0.001$; respectively).

RES C-Dots Prevent 6-OHDA-Induced Elevation of Caspase-3 mRNA levels in SH-SY5Y Cells

The mRNA expression of caspase-3 was markedly up-regulated in the 6-OHDA group compared with the control group ($p < 0.001$). The transcription level of caspase-3 at 12.5, 25, and 50 µg/mL concentrations of RES C-Dots were significantly decreased in SH-SY5Y cells compared with that of the 6-OHDA group (Figure 4) ($p < 0.05$, $p < 0.001$, and $p < 0.001$; respectively).

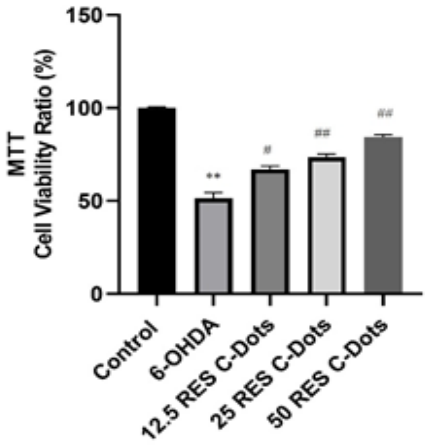

Figure 1. Effects of RES C-Dots on the SH-SY5Y cells viability. Findings are given as the means ± SD. ** p<0.001 vs control group, # p<0.05 vs 6-OHDA group, ## p<0.001 vs 6-OHDA group.

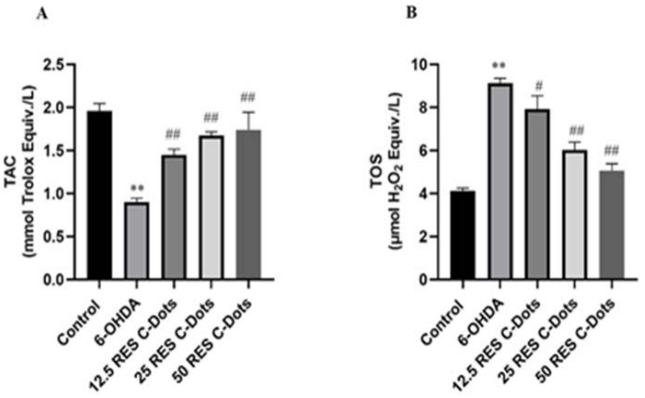

Figure 2. Effects of RES C-dots on the levels of TAS and TOS on SH-SY5Y cell. Findings are given as the means ± SD. ** p<0.001 vs control group, # p<0.05 vs 6-OHDA group, ## p<0.001 vs 6-OHDA group.

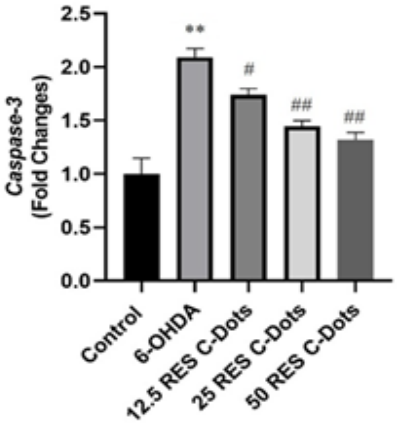

Figure 4. Effects of RES C-dots on the apoptosis of SH-SY5Y cells. Findings are given as the means ± SD. ** p<0.001 vs control group, # p<0,05 vs 6-OHDA group, ## p<0.001 vs 6-OHDA group.

Discussion

In this report, the neuroprotective effect of RES C-Dots was investigated in an in vitro PD model. 6-OHDA is a potent neurotoxin that enters DANs via the dopamine transporter, accumulates in the cell, and causes oxidative damage leading to cell death of DANs (12). In the present report, the protective effect of RES C-Dots on cell injury induced by 6-OHDA was investigated using an MTT assay. As expected, 200 µM 6-OHDA exposure significantly impaired the viability of SH -SY5Y cells, with viability as low as 51.4% (Figure 1). The results shown in this study are consistent with previous reports (12, 14).

Conversely, pretreatment with RES C-Dots significantly increased the viability of SH -SY5Y cells treated with 6-OHDA. The increased cell viability demonstrates the potential protective role of RES C-Dots against 6-OHDA, which leads to cell damage in SH -SY5Y cells. These results highlight the specificity of RES C-Dots in protecting neuronal cells from 6-OHDA-induced damage.

6-OHDA has been shown to cause neurotoxicity through the formation of free radicals that trigger oxidative damage in DANs (15). The imbalance in physiological maintenance of redox potential in DANs impedes various biological processes and eventually leads to neuronal death (16). Decreased total antioxidant levels and increased total oxidant levels are biochemical indicators of cellular damage (12). Therefore, we determined the levels of TAS and TOS to estimate the extent of oxidative damage in cells.

TAC is an analyte commonly used to evaluate the antioxidant status of biological samples and to assess the antioxidant response to free radicals generated in a given disease (17). TOS another parameter, TAC, is used to estimate the cumulative oxidative influences of various oxidants in biological systems (18). It is well known that 6-OHDA can generate free radicals that cause oxidative damage to SH -SY5Y cells by decreasing antioxidant activity and increasing intracellular oxidants, thereby impairing mitochondrial function, leading to neuronal apoptosis (14). it was shown that 6-OHDA both decreased TAC levels (Figure 2A) and increased TOS levels (Figure 2B) in SH -SY5Y cells compared with control. In support of our findings, 6-OHDA has been reported to cause oxidative damage by altering the balance between the antioxidant defense system in SH -SY5Y cells in favor of oxidants (19, 20).

Pretreatment with RES C-dots at all concentrations significantly increased TAC levels and markedly decreased levels of TOS, indicating attenuation of oxidative stress. Overall, the results indicate that RES C-dots has been shown to reduce oxidative stress induced by 6-OHDA in SH -SY5Y cells and excellently protect them from oxidative damage. Moreover, the results in Figure 2 were consistent with the cell viability assay (Figure 1), which demonstrated the specific protective effect of RES C-dots on 6-OHDA-induced damage in SH -SY5Y.

PD is characterized by DANs dysfunction which would be associated with persistent neuroinflammation. When levels of free radicals are augmented, these can activate pro-inflammatory pathways further perpetuating the detrimental environment for vulnerable neuronal cells (21). Therefore, targeting the inflammatory process has been recognized as a therapeutic target for Parkinson's disease (20). 6-OHDA was demonstrated to augment the levels of inflammatory cytokines including TNF-α and IL-6 in SH-SY5Y cells (22). Similar to the literature, in this study, increases in levels of TNF-α and IL-1β were found as a result of 6-OHDA administration. Figure 3 reveals that following pre-incubation of SH-SY5Y with RES C-dots, levels of TNF-α and IL-1β were remarkably diminished (Figure 3A-B). These findings suggested RES C-Dots may act as an anti-inflammatory agent for the management of the neurodegenerative processes in PD.

Apoptosis in DANs contributes seriously to movement disorders and death in PD patients. A considerable amount of documentation reported that 6-OHDA also instigates apoptosis via caspase activation following inflammation with excessive free radicals elevation (23, 24). Caspase-3 is a considerable component of the cysteine protease family in the mitochondrial apoptotic pathway (24). In this report, when cells were subjected to 6-OHDA, the mRNA expression of caspase-3 was markedly enhanced beckoning the 6-OHDA-induced apoptosis in SH-SY5Y cells, confirming previous studies (24, 25). In opposition, the RES C-Dots treatment at all concentrations down-regulated caspase-3 expression and showed antiapoptotic effects. RES C-Dots could prevent apoptosis of DANs and improve their resistance to 6-OHDA in vitro which demonstrated RES C-Dots exerted a protective impact by diminishing the apoptosis in PD.

Conclusion

The current study showed for the firstly time that C-dots synthesized from RES protect the viability of DANs from 6-OHDA toxicity by inhibiting oxido-inflammatory stress and apoptosis. Considering the low bioavailability of RES, these characteristics may make RES C-dots a feasibly potent new candidate for neuroprotection in PD.

Ethical Approval: There are no ethical issues regarding the publication of this study

Author Contributions:
Concept: B.Ç., B.D.
Literature Review: B.D.
Design : B.Ç., B.D.
Data acquisition: B.Ç., B.D.
Analysis and interpretation: B.Ç., B.D.
Writing manuscript: B.Ç., B.D.
Critical revision of manuscript: B.D.
Conflict of Interest: *The authors have no conflicts of interest..*
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