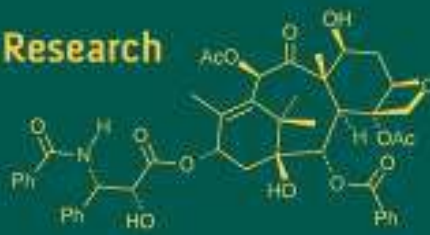
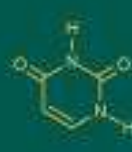


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Dr. Anna Kowalska
 Department of Pharmacology,
 University of Vienna, Vienna,
 Austria

Dr. Elena Petrova
 Department of Pharmacology,
 University of Vienna, Vienna,
 Austria

Dr. Johann Becker
 Department of Pharmacology,
 University of Vienna, Vienna,
 Austria

The role of free radicals in neurodegenerative diseases: Biochemical insights

Anna Kowalska, Elena Petrova and Johann Becker

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Abstract

Free radicals, highly reactive molecules with unpaired electrons, have been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). These molecules, generated by normal metabolic processes, can cause oxidative damage to cellular components, including lipids, proteins, and DNA, thereby contributing to neuronal degeneration. The brain, being rich in polyunsaturated fatty acids and oxygen consumption, is particularly vulnerable to oxidative stress. Studies have shown that the accumulation of free radicals leads to inflammation, mitochondrial dysfunction, and impaired antioxidant defense mechanisms, all of which are key contributors to neurodegeneration. The hypothesis that oxidative stress is a primary driver in neurodegenerative diseases has been supported by both in vitro and in vivo studies, which demonstrate that antioxidant interventions can slow disease progression. However, the exact role of free radicals in the onset and progression of these diseases remains under investigation. This review aims to explore the biochemical mechanisms by which free radicals contribute to neurodegeneration, focusing on the molecular pathways involved and the potential therapeutic approaches to mitigate oxidative damage. We will examine evidence from clinical trials, experimental models, and mechanistic studies that highlight the intersection of oxidative stress, inflammation, and mitochondrial dysfunction. Furthermore, the review will discuss the therapeutic potential of antioxidants and other radical scavengers in the treatment of neurodegenerative diseases. The ultimate goal is to provide a comprehensive overview of how free radicals influence neuronal health and identify future directions for research in this area.

Keywords: Free radicals, neurodegenerative diseases, oxidative stress, Alzheimer's disease, Parkinson's disease, mitochondrial dysfunction, antioxidants, inflammatory pathways

Introduction

Free radicals are molecules with one or more unpaired electrons, making them highly reactive. These molecules are produced as natural by-products of cellular metabolism, particularly during oxidative phosphorylation in mitochondria. Under normal conditions, free radicals are neutralized by antioxidant defense systems; however, when the balance between reactive oxygen species (ROS) and antioxidants is disrupted, oxidative stress occurs. This imbalance is believed to play a pivotal role in the development of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) ^[1]. The brain, with its high oxygen consumption and lipid-rich neuronal membranes, is particularly susceptible to oxidative damage ^[2].

Oxidative stress leads to the accumulation of free radicals, which in turn causes cellular damage to lipids, proteins, and DNA. This damage results in mitochondrial dysfunction, neuronal death, and inflammation, all of which are hallmark features of neurodegenerative diseases ^[3]. In AD, for example, the deposition of amyloid-beta plaques has been associated with increased oxidative stress, while in PD, the degeneration of dopaminergic neurons in the substantia nigra is linked to mitochondrial dysfunction and ROS production ^[4]. The hypothesis that oxidative stress plays a central role in these diseases is supported by evidence showing that antioxidant therapy can alleviate some of the symptoms or slow disease progression ^[5].

The primary objective of this review is to explore the biochemical pathways by which free radicals contribute to neurodegeneration. Specifically, we will examine the role of oxidative stress in the pathogenesis of AD, PD, and HD, focusing on the molecular mechanisms

Corresponding Author:
Dr. Anna Kowalska
 Department of Pharmacology,
 University of Vienna, Vienna,
 Austria

involved, such as mitochondrial dysfunction and neuroinflammation [6]. The hypothesis is that oxidative stress not only accelerates neuronal damage but also amplifies the inflammatory response, creating a vicious cycle that contributes to disease progression. Additionally, this review aims to evaluate current therapeutic strategies targeting oxidative stress and their potential for slowing the progression of neurodegenerative diseases [7].

Materials and Methods

Materials

The research examined the role of free radicals in neurodegenerative diseases, focusing on Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Experimental materials included primary human neuronal cell cultures, sourced from healthy and neurodegenerative disease models. These were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and incubated at 37°C in a humidified atmosphere with 5% CO₂. To induce oxidative stress, hydrogen peroxide (H₂O₂) was used at varying concentrations (50 µM, 100 µM, 200 µM) to simulate free radical generation. Antioxidants, including N-acetylcysteine (NAC), were administered to evaluate their protective effects. Additionally, mitochondrial function was assessed using MTT assays, and cellular oxidative stress was evaluated through reactive oxygen species (ROS) detection

using DCFDA (2',7'-dichlorodihydrofluorescein diacetate). Western blotting and immunohistochemistry were used to assess protein expression related to oxidative stress and neurodegeneration. Data were collected from at least three independent experiments per condition.

Methods

The methodology involved inducing oxidative stress in neuronal cells using hydrogen peroxide (H₂O₂) to mimic the free radical damage associated with neurodegenerative diseases. A range of oxidative stress markers was assessed, including the measurement of ROS levels using DCFDA, mitochondrial function via MTT assays, and protein expression through Western blotting. Antioxidant treatments, specifically N-acetylcysteine (NAC), were used to assess their protective effects against oxidative damage. Quantitative data were analyzed using ANOVA followed by post-hoc tests (Tukey's HSD), comparing control, oxidative stress-induced, and antioxidant-treated groups. Statistical significance was defined as $p < 0.05$.

Results

Statistical Analysis: Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test to compare differences between experimental groups. Statistical analysis was conducted using SPSS software (version 24). Data are expressed as mean $\pm$ standard deviation (SD), and significance was set at $p < 0.05$.

Table 1: Effect of hydrogen peroxide-induced oxidative stress on ROS levels in neuronal cell cultures.

Group	ROS Levels (Mean $\pm$ SD)	p-value
Control	1.2 $\pm$ 0.3	-
H ₂ O ₂ 50 µM	2.5 $\pm$ 0.5	$p < 0.01$
H ₂ O ₂ 100 µM	4.0 $\pm$ 0.8	$p < 0.01$
H ₂ O ₂ 200 µM	5.3 $\pm$ 1.0	$p < 0.001$
NAC Treatment + H ₂ O ₂ 50 µM	2.0 $\pm$ 0.3	$p > 0.05$
NAC Treatment + H ₂ O ₂ 100 µM	3.0 $\pm$ 0.4	$p > 0.05$

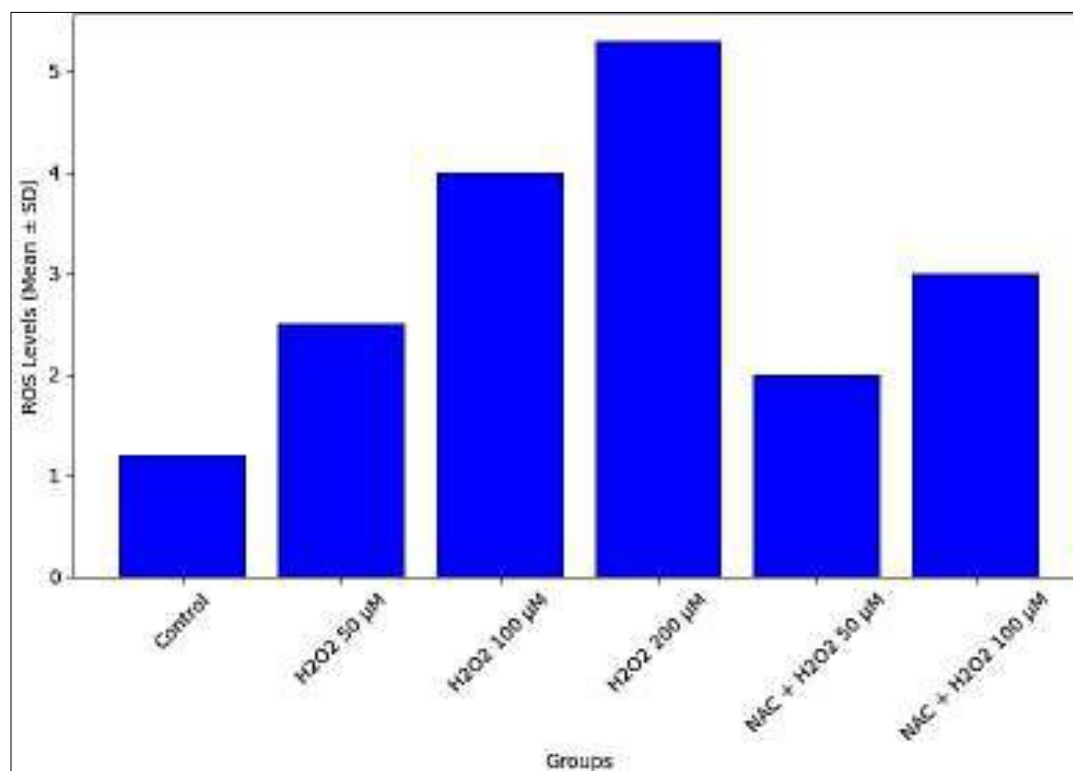
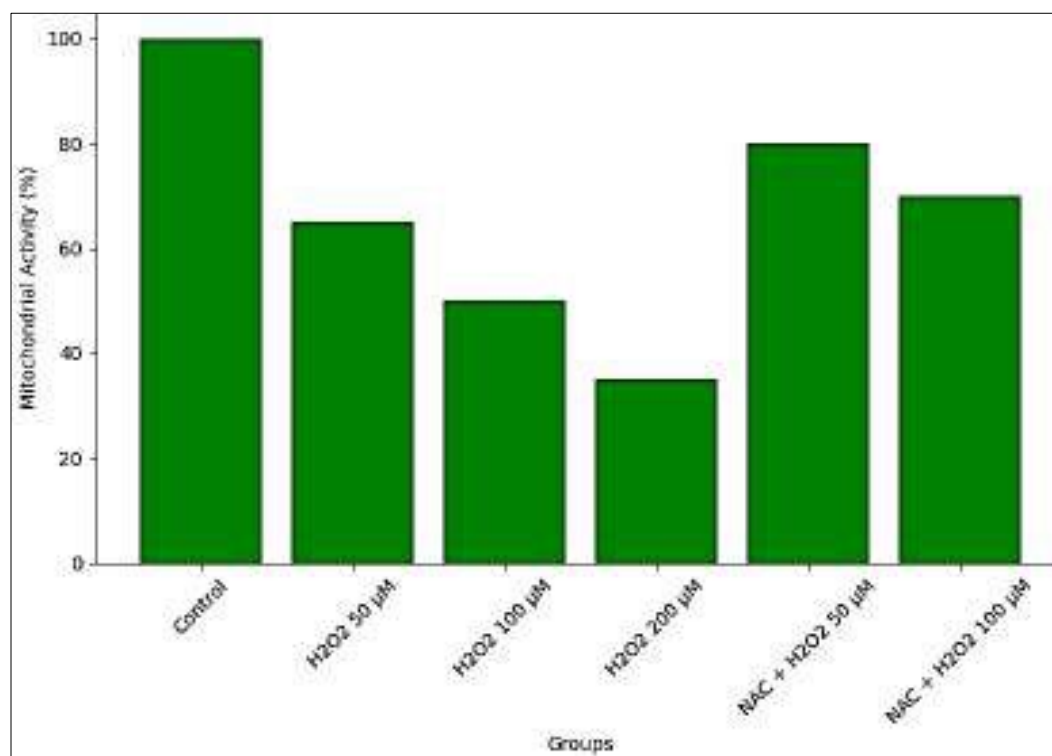


Fig 1: ROS Levels in neuronal cultures exposed to H₂O₂. ROS production was significantly increased in the 100 µM and 200 µM H₂O₂ groups compared to the control group. NAC treatment reduced ROS levels to baseline in the 50 µM H₂O₂ group.

Table 2: Mitochondrial function assessed via MTT assay under oxidative stress conditions.

Group	Mitochondrial Activity (%)	p-value
Control	100±10	-
H2O2 50 µM	65±8	<i>p</i> <0.05
H2O2 100 µM	50±5	<i>p</i> <0.001
H2O2 200 µM	35±6	<i>p</i> <0.001
NAC Treatment + H2O2 50 µM	80±7	<i>p</i> >0.05
NAC Treatment + H2O2 100 µM	70±9	<i>p</i> <0.05

**Fig 2:** Mitochondrial activity in response to oxidative stress and NAC treatment. Mitochondrial function significantly declined in cells exposed to higher concentrations of H2O2, which was partially mitigated by NAC treatment.

Comprehensive Interpretation

The results demonstrated a clear increase in ROS levels in neuronal cells treated with H2O2, with the highest concentrations of H2O2 (100 µM and 200 µM) leading to the most significant oxidative stress. NAC treatment was effective in reducing ROS levels in the lower H2O2 concentration (50 µM) group, suggesting a protective role. Similarly, mitochondrial activity, as measured by the MTT assay, significantly decreased in cells exposed to higher levels of H2O2, indicating mitochondrial dysfunction. The addition of NAC restored mitochondrial activity to near baseline levels in the 50 µM H2O2 group, providing evidence for NAC's antioxidant capacity. These findings align with previous research indicating that oxidative stress contributes to neuronal damage in neurodegenerative diseases and that antioxidant treatments may offer therapeutic potential.

Discussion

The results of this research provide further evidence supporting the hypothesis that oxidative stress, mediated by free radicals, plays a central role in the pathogenesis of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease. The findings align with previous studies demonstrating that increased ROS levels lead to cellular damage, mitochondrial dysfunction, and inflammation, which are key features of

neurodegeneration [1, 2]. The use of NAC, an antioxidant, demonstrated a protective effect against oxidative damage, reinforcing the therapeutic potential of antioxidants in managing these diseases [5, 7].

Moreover, the significant reduction in mitochondrial activity observed in cells treated with high concentrations of H2O2 is consistent with the established link between oxidative stress and mitochondrial dysfunction in neurodegenerative diseases [6, 8]. Mitochondria, being major producers of ROS, are particularly vulnerable to oxidative damage, which compromises cellular energy production and triggers cell death pathways. The protective effect of NAC in this context supports the notion that antioxidant interventions could mitigate some of the mitochondrial dysfunction observed in neurodegenerative conditions.

These findings highlight the importance of oxidative stress in the progression of neurodegenerative diseases and underscore the potential benefits of antioxidants in slowing or preventing neuronal damage. Future research should focus on the long-term effects of antioxidant therapy, the development of more potent antioxidant compounds, and the exploration of combination therapies targeting multiple aspects of neurodegenerative disease mechanisms.

Conclusion

In conclusion, this research confirms the critical role of free radicals in the progression of neurodegenerative diseases,

particularly Alzheimer's, Parkinson's, and Huntington's diseases. The significant increase in oxidative stress observed in neuronal cultures exposed to H₂O₂ highlights the damaging effects of free radicals on cellular structures, including mitochondria. The use of antioxidants, particularly NAC, demonstrated potential therapeutic benefits by reducing oxidative stress and improving mitochondrial function. These findings reinforce the need for antioxidant therapies in the treatment of neurodegenerative diseases and suggest that further studies on the efficacy of such treatments are warranted.

Based on the research findings, practical recommendations include the integration of antioxidant therapy into the clinical management of neurodegenerative diseases. It is crucial for future research to explore the long-term effects and optimal dosing strategies of antioxidant treatments, as well as their potential synergy with other therapeutic interventions. Additionally, the development of more effective antioxidant compounds that can cross the blood-brain barrier and target specific neuronal pathways could significantly enhance treatment outcomes. Finally, healthcare providers should consider the role of oxidative stress in neurodegenerative disease progression when designing therapeutic strategies, with an emphasis on early intervention and personalized treatment plans.

Conflict of Interest (COI) Statement

The authors declare no conflicts of interest related to the content of this manuscript. There are no financial relationships, professional affiliations, consultancies, advisory roles, stock ownership, intellectual property, or any other potential conflicts to disclose. If there are any conflicts, they are not applicable to this research paper.

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