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The effect of sulforaphane against glutamate-induced oxidative stress and inflammation in SH-SY5Y cells

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ABSTRACT

Glutamate (GLT) induced excitotoxicity plays a central role in the pathogenesis of various neurodegenerative diseases through mechanisms involving oxidative stress and inflammation. Sulforaphane (SFN), a natural isothiocyanate derived from cruciferous vegetables, has demonstrated neuroprotective effects. This study aimed to investigate the protective effects of SFN against glutamate-induced oxidative stress and inflammation in SH-SY5Y neuroblastoma cells. SH-SY5Y cells were divided into four experimental groups: (1) Control, (2) SFN (5 μM SFN for 30 min), (3) GLT (10 mM glutamate for 24 h), and (4) GLT + SFN (pre-treatment with SFN followed by glutamate exposure). TAS and TOS levels, and pro-inflammatory cytokine expression (TNF-α and IL-1β) were evaluated using standard biochemical assays. GLT exposure significantly increased TOS and pro-inflammatory cytokine levels, reducing TAS levels. Pretreatment with SFN reduced the levels of TOS and inflammatory markers compared to the GLT group, and increased the levels of TAS.

SFN exerts a protective effect against GLT-induced cytotoxicity in SH-SY5Y cells, likely through its antioxidative and anti-inflammatory properties. These findings suggest that SFN may have therapeutic potential in neurodegenerative disease models.

Keywords: Glutamate, Sulforaphane, SH-SY5Y cells, Excitotoxicity, Oxidative stress

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INTRODUCTION

Glutamate excitotoxicity, a pathological process induced by excessive activation of glutamate receptors, is one of the primary mechanisms implicated in the progression of various neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [1, 2]. Under physiological conditions, glutamate serves as the major excitatory neurotransmitter in the central nervous system. However, when present at supraphysiological concentrations, it leads to sustained calcium influx, mitochondrial dysfunction, and excessive production of reactive oxygen species (ROS), ultimately triggering oxidative stress, inflammation, and neuronal cell death [3].

The human neuroblastoma SH-SY5Y cell line is a widely used in vitro model to study neuronal damage and protection mechanisms due to its dopaminergic properties and high susceptibility to glutamate-induced cytotoxicity [4-6]. In this model, glutamate-induced toxicity has been associated with both oxidative damage and activation of pro-inflammatory signalling cascades, particularly those involving NF-κB and mitogen-activated protein kinases [7]. These insights have prompted increasing interest in identifying natural compounds with antioxidant and anti-inflammatory potential that could confer neuroprotection.

Sulforaphane (SFN), a naturally occurring isothiocyanate derived from cruciferous vegetables such as broccoli, has drawn considerable attention due to its potent cytoprotective effects. SFN is best known as an activator of the nuclear factor erythroid 2-related factor 2 signalling pathway, which regulates the expression of a battery of detoxifying and antioxidant enzymes, including heme oxygenase-1, glutathione S-transferases, and NAD(P)H: quinone oxidoreductase 1 [8]. By upregulating these endogenous antioxidant defences, SFN effectively reduces intracellular oxidative stress levels and prevents apoptotic cell death [9]. Moreover, SFN exhibits anti-inflammatory properties by inhibiting the activation of NF-κB and attenuating the expression of pro-inflammatory cytokines such as TNF-α and IL-1β [10].

This investigation contributes to the growing body of evidence supporting the neurotherapeutic potential of SFN. It provides mechanistic insights into its role in combating neurotoxicity and neuroinflammation, thereby highlighting its possible translational value in neurodegenerative disease treatment.

METHODS

Cell Culture and Experimental Groups

SH-SY5Y cells, widely utilised in neurotoxicity research, were procured from the American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's Modified Eagle Medium [DMEM)/F-12 HAM nutrient medium (1:1)] supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained at 37 °C in a humidified CO_2 incubator. For experimental procedures, cells were seeded into 25 cm^2 culture flasks and allowed to adhere for 24 hours. GLT and SFN were sourced from Sigma-Aldrich (St. Louis, MO, USA).

The study was conducted in 4 groups.

Control group: No treatment was applied to this group.

SFN group: The cells were treated with SFN (dissolved in DMSO; DMSO was utilized at a final concentration of 0.01%) at 5 μM for 24 h [11].

GLT group: 10 mM glutamate was added and incubated for 24 h [12].

GLT+SFN group: The SH-SY5Y cells were treated with SFN chrysin 5 μM 30 min prior to glutamate exposure and incubated for 24 h.

Measurement of Biochemical Parameters in SH-SY5Y Cells

The samples were then centrifuged at 1000 rpm for 20 minutes according to the respective kit protocols. Following centrifugation, the supernatants were discarded, and the resulting cell pellets were resuspended in phosphate-buffered saline (PBS; pH 7.4) to achieve a final concentration of approximately 1×10^6 cells/mL. Cell lysis was performed to release intracellular contents, utilizing repeated freeze-thaw cycles. Total protein content was quantified using the Bradford assay, which is based on the principle that Coomassie Brilliant Blue G-250 dye exhibits a shift in absorbance and develops a blue colour of varying intensities depending on the protein concentration in an acidic environment. The levels of TAS (Total Antioxidant Status), TOS (Total Oxidant Status), TNF-α, and IL-1β in the SH-SY5Y cell supernatants were quantified using ELISA kits obtained from Bioassay Technology Laboratory (China). Absorbance readings were recorded using a BioTek ELx808™ microplate reader by the manufacturer's instructions.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). For statistical analysis, one-way analysis of variance (ANOVA) was employed to assess differences among groups with normally distributed data, utilising SPSS statistical software. Tukey's post hoc test was applied when significant differences were detected to identify pairwise group differences. Statistical significance was defined as $p < 0.05$ for all analyses.

RESULTS

Modulatory effect of SFN on TAS and TOS levels on GLT-induced excitotoxicity in SH-SY5Y cells

To investigate the impact of SFN on oxidative stress in SH-SY5Y cell lysates, ELISA kits were utilised to quantify TAS and TOS. Exposure to 10 mM glutamate significantly reduced TAS levels compared to the untreated control group ($p < 0.05$). However, co-treatment with SFN following glutamate exposure led to a marked elevation in TAS levels relative to the GLT group ($p < 0.05$, Figure 1).

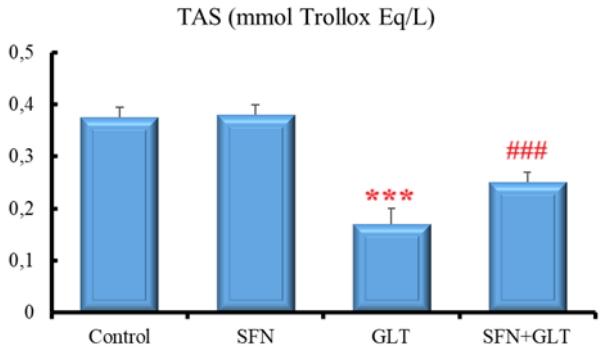

Figure 1. *Effect of SFN on TAS level in SH-SY5Y cells after GLT-induced cytotoxicity. (***p <0.05 compared to Control and SFN groups, ###p <0.05 compared to Control, SFN and GLT groups).*

Regarding TOS measurements, GLT exposure (10 mM) resulted in a significant increase in TOS levels when compared to the control group ($p < 0.05$). Notably, cells co-treated with SFN and GLT exhibited TOS levels comparable to the control, with no statistically significant difference observed. These findings indicate that SFN treatment mitigated the GLT-induced oxidative imbalance by enhancing TAS and reducing TOS levels ($p < 0.05$, Figure 2).

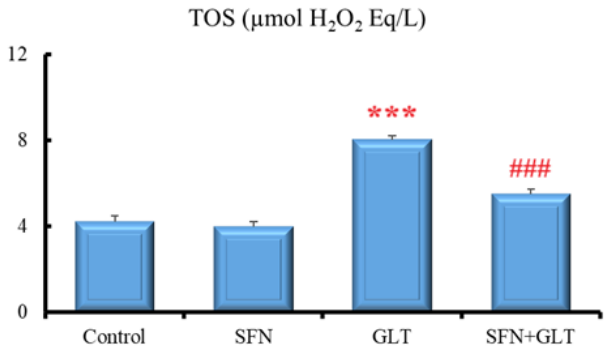

Figure 2. *Effect of SFN on TOS level in SH-SY5Y cells after GLT-induced cytotoxicity. (***p <0.05 compared to Control and SFN groups, ###p <0.05 compared to Control, SFN and GLT groups).*

Modulatory Effect of Chrysin on TNF-α and IL-1β Levels on GLT-induced excitotoxicity in SH-SY5Y cells

To assess the influence of SFN on pro-inflammatory cytokine expression, TNF-α levels were quantified using a commercial ELISA kit in SH-SY5Y cells exposed to GLT with or without SFN treatment. The data revealed a statistically significant elevation in TNF-α concentrations in the GLT-treated group compared to both the untreated control and SFN groups ($p < 0.05$). Conversely, co-treatment with SFN significantly attenuated the GLT-induced increase in TNF-α levels ($p < 0.05$, Figure 3).

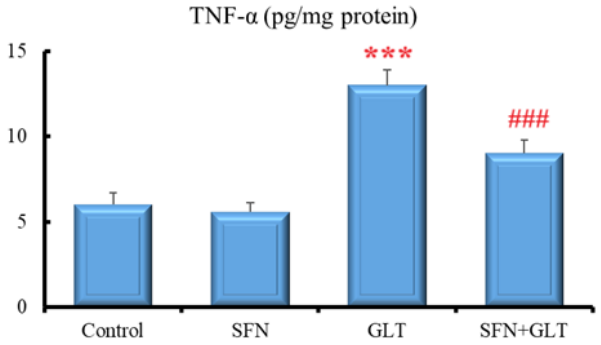

Figure 3. *Effect of SFN on TNF-α level in SH-SY5Y cells after GLT-induced cytotoxicity. (***p <0.05 compared to Control and SFN groups, ###p <0.05 compared to Control, SFN and GLT groups).*

Similarly, the effect of SFN on IL-1β levels was evaluated under GLT-induced excitotoxic conditions using a specific ELISA assay. The results demonstrated a marked increase in IL-1β levels in the GLT group relative to the Control and SFN groups ($p < 0.05$). Notably, IL-1β expression was significantly reduced in the GLT+SFN group compared to the GLT group

alone ($p < 0.05$), suggesting that SFN exerts an anti-inflammatory effect by downregulating IL-1β production under excitotoxic conditions (Figure 4).

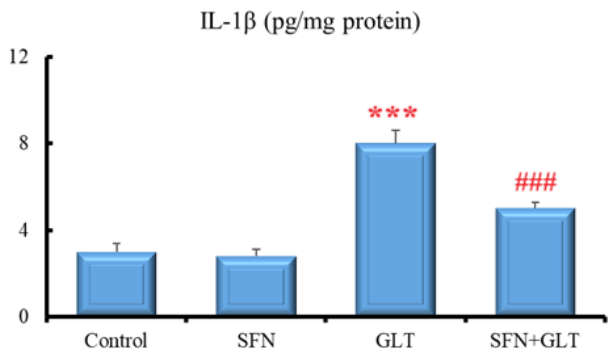

Figure 4. *Effect of SFN on IL-1β level in SH-SY5Y cells after GLT-induced cytotoxicity. (***p <0.05 compared to Control and SFN groups, ###p <0.05 compared to Control, SFN and GLT groups).*

DISCUSSION

The findings of this study demonstrate that sulforaphane (SFN) confers significant protection against glutamate-induced oxidative stress and inflammation in SH-SY5Y neuroblastoma cells. Exposure to glutamate led to marked cellular damage characterised by increased production of ROS, and elevated levels of pro-inflammatory cytokines TNF-α and IL-1β. These results are in agreement with previous studies, which have established glutamate excitotoxicity as a key driver of neuronal death in several neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [1, 13, 14]

Glutamate-induced cytotoxicity in neuronal cells involves complex mechanisms. Among these, mitochondrial dysfunction plays a critical role, leading to excessive ROS generation, membrane potential collapse, ATP depletion, and activation of apoptotic cascades [15]. Additionally, glutamate has been shown to activate microglial and astrocytic responses, further amplifying inflammatory signals and exacerbating neuronal damage [7]. In the present study, the observed upregulation of TNF-α and IL-1β suggests that inflammation plays a central role in glutamate-mediated neuronal toxicity.

Pre-treatment with SFN effectively reversed many of these pathological changes. SFN-treated cells exhibited significantly lower ROS levels and cytokine expression than the glutamate-only group, suggesting a robust antioxidant and anti-inflammatory effect. These findings are in line with the established role of SFN as a potent activator of the Nrf2 signaling pathway. Upon activation, Nrf2 translocates to the nucleus. It binds to antioxidant response elements (ARE) to induce the transcription of several cytoprotective genes, including heme oxygenase-1, NAD(P)H: quinone oxidoreductase 1, and glutathione peroxidase [16, 17]. The upregulation of these enzymes enhances the cellular redox capacity and protects against oxidative insults.

Moreover, SFN has been shown to inhibit the nuclear factor kappa B (NF-κB) pathway, a major regulator of inflammation. NF-κB activation results in the transcription of several pro-inflammatory genes, including TNF-α, IL-1β, COX-2, and iNOS [18]. Inhibition of this pathway by SFN likely contributes to the decreased expression of TNF-α and IL-1β observed in our study [19]. This dual action, simultaneously targeting oxidative stress and inflammation, makes SFN a desirable candidate for neuroprotective therapy.

Importantly, our results also provide insights into the timing and dosing of SFN intervention. The administration of SFN (5 μM) 30 min. prior to glutamate exposure was sufficient to elicit significant cytoprotective effects, suggesting that early antioxidant intervention may open a critical window for therapeutic efficacy. Previous studies have also indicated that low micromolar concentrations of SFN can induce cytoprotective effects without causing cytotoxicity in neuronal and glial cell lines [20, 21].

Nonetheless, while the current study offers promising data, it has limitations. The SH-SY5Y cell line, although widely used as a dopaminergic neuron model, does not fully mimic the complexity of mature neurons or the in vivo neural microenvironment. Moreover, the pathways underlying SFN-mediated protection were not directly confirmed via Nrf2 or NF-κB inhibition assays. Future studies utilizing gene silencing or pharmacological inhibitors would be necessary to confirm the precise molecular mechanisms involved. Additionally, extending this work to in vivo models of neurodegeneration could help validate the translational potential of SFN.

CONCLUSION

Our study highlights the protective role of sulforaphane in mitigating glutamate-induced neurotoxicity through the attenuation of oxidative stress and inflammatory responses. These findings suggest that SFN, as a dietary-derived compound, holds considerable promise as a neuroprotective agent, especially in diseases characterised by glutamate excitotoxicity and redox imbalance. Further mechanistic and translational studies are warranted to elucidate its therapeutic potential fully.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Conflicts of Interest

There is no conflict of interest for the publication of this article.

Disclosure

The authors have reported no conflicts of interest in preparing and publishing this article.

Ethics committee approval

This research was carried out using cells propagated through commercially available cell culture. Ethics committee approval is not required in this study. The study was conducted following the international declaration, guidelines, etc.

Referee Evaluation Process

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