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Sugammadex Causes C6 Glial Cell Death and Exacerbates Hydrogen Peroxide-Induced Oxidative Stress

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Research Article

History

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ABSTRACT

Objective: Sugammadex (SUG) quickly reverses steroidal neuromuscular blocking drugs after anesthesia. It has been reported that SUG has a toxic effect on neurons in primary culture. This study aims to examine the effect of SUG on glial cell viability, oxidative stress, and apoptosis in C6 glial cells after exposure to H_2O_2.
Method: In this study, C6 glioma cell line was used to study the effect of SUG on the glial cell in four cell groups. The control group was untreated. Cells in the H_2O_2group were treated with 0.5 mM H_2O_2 for 24 h. Cells in the SUG group were treated with 50 µg/mL SUG for 24 h. Cells in the SUG+ H_2O_2 group were pre-treated with 50 µg/mL of SUG for 1 h before 24-h exposure to 0.5 mM H_2O_2. Cell viability was evaluated using XTT assay. Total antioxidant status (TAS), total oxidant status (TOS), caspase-3, Bax, 8-hydroxy-2' -deoxyguanosine (8-OHdG), and cleaved-PARP levels in the cells were measured by commercial kits.
Results: SUG significantly decreased the viability of C6 cells after H_2O_2-induced oxidative stress ($p < 0.05$). SUG pretreatment also raised TOS levels and led to increased Bax, Caspase-3, 8-OHdG, and cleaved PARP levels after H_2O_2-induced oxidative damage in C6 cells ($p < 0.05$).
Conclusions: SUG is toxic agent on neurons and exacerbates H_2O_2-induced oxidative damage in C6 cells.

***Keywords**: Suggammadex, cell death, C6 glioma*

Sugammadex, C6 Glial Hücrelerin Ölümüne Yol Açar ve Hidrojen Peroksitin Indüklediği Oksidatif Stresi Şiddetlendirir

Süreç

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ÖZ

Amaç: Sugammadex (SUG), anesteziden sonra steroidal nöromüsküler bloke edici ilaçları hızla tersine çevirir. SUG'nin primer nöron kültüründe toksik etkisi olduğu bildirilmiştir. Bu çalışma, SUG'nin H_2O_2'ye maruz kaldıktan sonra C6 glial hücrelerinde hücre canlılığı, oksidatif stres ve apoptoz üzerindeki etkisini incelemeyi amaçlamaktadır.
Yöntem: Bu çalışmada, SUG'nin glial hücreler üzerindeki etkisini incelemek için C6 glioma hücre hattı kullanıldı. Kontrol grubu tedavi edilmeyen gruptu. H_2O_2 grubundaki hücreler, 24 saat boyunca 0,5 mM H_2O_2 ile muamele edildi. SUG grubundaki hücreler, 24 saat boyunca 50 µg/mL SUG ile tedavi edildi. SUG+ H_2O_2 grubundaki hücreler, 0,5 mM H_2O_2'ye 24 saat maruz kalmadan önce 1 saat boyunca 50 µg/mL SUG ile ön işleme tabi tutuldu. Hücre canlılığı, XTT yöntemi kullanılarak değerlendirildi. Hücrelerdeki toplam antioksidan durumu (TAS), toplam oksidan durumu (TOS), kaspaz-3, Bax, 8-hidroksi-2'-deoksiguanozin (8-OHdG) ve bölünmüş-PARP seviyeleri ticari kitler ile ölçüldü.
Bulgular: SUG, C6 hücrelerinin canlılığını önemli ölçüde azalttı ($p < 0.05$). SUG ön tedavisi ayrıca TOS seviyelerini yükseltti ve C6 hücrelerinde H_2O_2 ile indüklenen oksidatif hasardan sonra Bax, Caspase-3, 8-OHdG ve parçalanmış-PARP'ın artmış seviyelerine yol açtı ($p < 0.05$).
Sonuç: SUG, nöronlar üzerinde toksik ajandır ve C6 hücrelerinde H_2O_2 kaynaklı oksidatif hasarı şiddetlendirir.

***Anahtar sözcükler**: Sugammadeks, hücre ölümü, C6 glioma.*

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Introduction

Neuromuscular blocking (NMB) agents are widely used to provide skeletal muscle relaxation for endotracheal intubation and artificial ventilation, as well as patient immobility to achieve optimal surgical conditions[1]. However, postoperative residual NMB may cause respiratory complications, airway blockage, and decreased oxygen saturation[2]. In this regard, the pharmacological reversal of NMB is an important tool in the prevention of postoperative complications. Sugammadex (SUG) is a modified γ-cyclodextrin that terminates neuromuscular blockade by binding selectively with amino steroid NMB agents such as rocuronium or vecuronium[3]. Depending on the dosage, SUG may reverse a moderate or deep NMB with no muscular weakness[4].

Previous clinical studies have demonstrated that SUG is an effective and safe agent for the fast reversal of amino steroid NMB[5-7]. Further, since SUG does not have an anticholinesterase effect and does not require atropine, it offers more cardiovascular stability than neostigmine (commonly used reversal method)[8]. Furthermore, when it comes to NMB reversal after surgery, SUG is three to eight times faster than neostigmine[9]. On the other hand, SUG has some undesirable side effects, such as parasomnia, paresthesia, bradycardia, tachycardia, cough, temperature changes, abdominal discomfort, and dizziness[10]. There are also studies reporting that SUG itself causes hypersensitivity reactions[11]. Moreover, one study has shown that the recommended doses of SUG may result in neuron death in primary cultures[12]. Although this is unlikely in an intact brain barrier, SUG may have adverse effects on neuronal cells if the blood-brain barrier permeability is altered by trauma, systemic infection, neurodegenerative diseases, or immature nervous system[13-16].

Although there are a large number of studies on the impact of SUG on other organ systems, there are a limited number of research on its effects on the nervous system. Clinically relevant SUG doses are known to lead to neuronal toxicity, but the underlying mechanisms remain unclear. In the current study, we examined the effect of SUG on H_2O_2-induced oxidative stress in C6 glial cells and levels of caspase-3, Bax, 8-hydroxy-2′ -deoxyguanosine (8-OHdG), and cleaved PARP.

Material and Methods

Cell Culture and Chemicals

C6 glioma (CRL107) cell lines were purchased from American Type Culture Collection and cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, Altrincham, UK) including 1% L-glutamine (Sigma-Aldrich Co., St Louis, MO, USA), 10% Fetal Bovine Serum (Sigma- Aldrich Co., St Louis, MO, USA), and 1% penicillin/streptomycin (Sigma-Aldrich Co., St Louis, MO, USA). The cells were kept at 37°C in the air humidified by 5% CO2. SUG and H_2O_2 (Sigma-Aldrich Co., St Louis, MO, USA) were dissolved in DMEM.

Cell Viability Assay

XTT assay (Roche Diagnostic, MA, USA) was used to assess cell viability as previously described[17]. C6 glioma cells were seeded in 96-well-plates containing 100-uL DMEM with a cell density of 1 x 10^4 per well and left to grow overnight before adding SUG. The effect of SUG of C6 glioma cell viability was investigated in four groups. The control group was not treatment with either H_2O_2 or SUG. Cells in the H_2O_2 group were treated with 0.5 mM H_2O_2 for 24 h[18]. After the preliminary dose study, 50 µg/mL was chosen as the appropriate dose[12]. Cells in the SUG group were treated with 50 µg/mL of SUG for 24 h. Cells in the SUG+H_2O_2 group were pre-treated with 50 µg/mL of SUG for 1 h before exposure to 0.5 mM H_2O_2 for 24 h. The medium was removed after incubation and each well was washed twice with phosphate buffered saline (PBS). At the final stage, 100 µL DMEM and a mixture of 50 µL XTT labeling solution were added, and then the plates were kept at 37 ° C for 4 h. After the plates are shake, the absorbans was determined by an ELISA microplate reader in 450 nm (Thermo Fisher Scientific, Altrincham, UK). All experiments were conducted three times and cell vitality was measured as a percentage of the amount of living cells compared to control cells.

Preparation of Cell Homogenates

Preparation of cell homogenates was carried out as previously described[19]. The cells in each group were sampled in sterile tubes and then centrifuged for about 10 minutes at 2000 rpm. After removal of the supernatants, the cell pellets were suspended in PBS (pH 7.4) to obtain cell suspension with a concentration of about 1 million/ml. Cells disintegrated through successive freezing-thaw cycles, allowing internal components to come out. They were centrifuged at 4000 rpm for 10 min at a temperature of 4°C. Then, the supernatants were obtained for biochemical analysis. Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) was used to determine the total protein levels in the samples.

Measurement of Total Antioxidant Status (TAS) And Total Oxidant Status (TOS)

TAS levels in the supernatants of the cells were measured through commercial kits (Rel Assay, Antep, Turkey). The experiments were conducted according to the manufacturer's guidelines that were previously developed by Erel[20]. Standards of kit and cell supernatants premixed with reaction reagent (reagent I) were added into the wells. Then, staining reagent (reagent II) was added and incubated for 5 min at 37°C. After incubation, the absorbance was read at 660 nm. The method measures the reaction rate of free radicals by quantitating the absorbance of colored dianisidyl radicals formed during free radical reactions, which happen simultaneously with the production of hydroxyl radicals in Fenton reaction. The results were expressed as

micromolar Trolox equivalents per milligram tissue protein (µmol Trolox Eq/mg protein).

TAS levels in the supernatants of the cells were measured through commercial kits (Rel Assay, Antep, Turkey). The experiments were conducted according to the manufacturer's guidelines that were previously developed by Erel[21]. Standards of kit and cell supernatants premixed with reaction reagent (reagent I) were added into the wells. Then, staining reagent (reagent II) was added and incubated for 5 min at 37°C. After incubation, the absorbance was read at 530 nm. Since ferrous ion is oxidized to ferric ion when adequate quantities of oxidants are available in the medium, the method quantifies TOS levels by measuring the ferric ions in the samples with the use of xylenol orange. H_2O_2 was used for the calibration of the assay[21]. Therefore, the results of the assay were expressed in micromolar H_2O_2 equivalents per milligram tissue protein (µmol H_2O_2 Eq/mg protein).

Measurement of Caspase-3, Bax, 8-Ohdg and Cleaved PARP

Caspase-3, Bax, 8-OHdG and fragmented PARP levels in cell supernatants were measured with rat ELISA commercial kits (YL Biont, Shanghai, China). The experiments were conducted according to the manufacturer's instructions. In brief, standard and tissue samples were added into the wells and incubated for 60 min at 37°C. Following the washing phase, dyeing solutions were added and incubated for 15 minutes at 37°C. The stop solution was added and absorbance was read at 450 nm. Standard curves were plotted to determine the value of samples. The coefficients of variation within and between plates were <10%.

Statistical Analysis

The results were expressed as a mean ± standard error of the mean. The data analyses were performed with SPSS Version 25.0 for Windows. The statistical significance of the differences was evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Tukey multiple comparison test. $p < 0.05$ was considered statistically significant.

Results

Effect of SUG on Cell Survival After H_2O_2-Induced Oxidative Stress

In this study, The XTT cell proliferation test was conducted to assess the protective effects of SUG against oxidative damage induced by H2O2 in C6 cells. As shown in Figure1, preincubating the C6 cells with H_2O_2 for 24 hours significantly reduced cell survival as compared with control cells ($p < 0.05$). Similarly, SUG decreased cell survival in C6 cells as compared with untreated cells of the control. ($p < 0.05$). Moreover, the level of cell survival in the SUG+H_2O_2 group was lower than those in control, H_2O_2, and SUG groups, and the difference was statistically significant ($p < 0.05$).

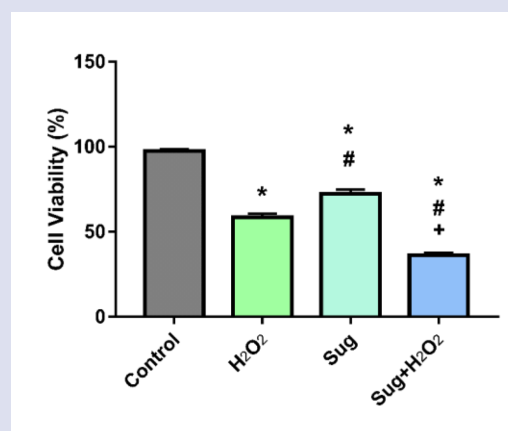
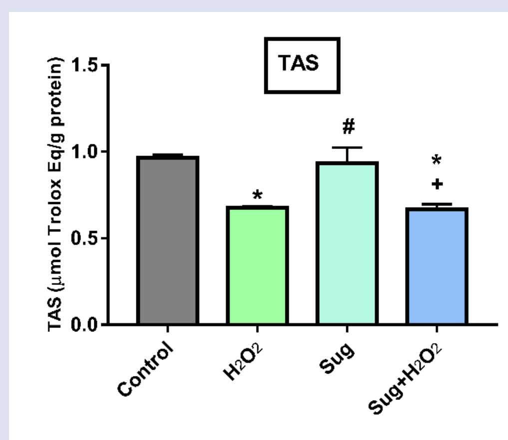


Figure 1. Effect of SUG on C6 cell survival after H2O2-induced oxidative damage. Values are presented as mean ± SEM. *p<0.05 vs Control group; #p<0.05 vs H2O2 group; +p<0.05 vs SUG group.



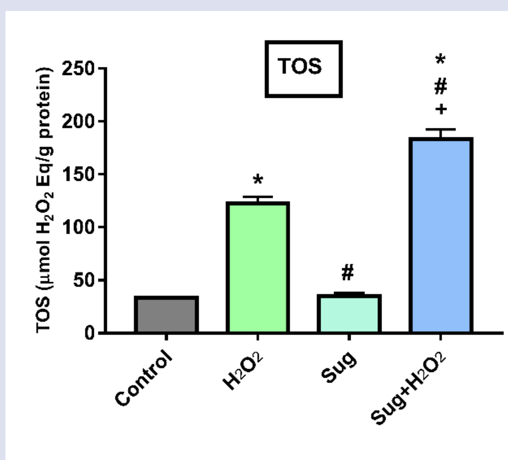


Figure 2. Effect of SUG on TAS and TOS levels in C6 cells after H2O2 -induced oxidative damage. Values are presented as mean ± SEM. *p<0.05 vs Control group; #p<0.05 vs H2O2 group; +p<0.05 vs SUG group.

Effect of SUG On TAS and TOS Levels After H_2O_2-Induced Oxidative Damage

As shown in Figure 2, the treatment of H_2O_2 alone or combined with SUG led to decreased TAS levels and increased TOS levels in C6 cells as compared with control cells ($p < 0.05$). On the other hand, SUG treatment alone caused increased TAS levels and decreased TOS levels in C6 cells ($p < 0.05$).

Effect of SUG On Caspase-3, Bax, 8-Ohdg, and Cleaved PARP Levels After H_2O_2-Induced Oxidative Damage

The ELISA assays were also conducted to evaluate the effects of SUG on apoptosis and oxidative DNA damage markers after H_2O_2-induced in C6 cells. As shown in Figure 3, the treatment of H_2O_2 increased Bax and Caspase-3 levels in C6 cells as compared with control cells. Moreover, the combination SUG and H_2O_2 significantly increased Bax and Caspase-3 levels in C6 cells compared to other groups ($p < 0.05$). Similarly, preincubating the C6 cells with H_2O_2 significantly increased 8-OHdG and cleaved PARP levels as compared with untreated cells of the control group ($p < 0.05$; Figure 4). Further, 8-OHdG and cleaved PARP levels were significantly higher in the SUG + H_2O_2 group than the H_2O_2 group ($p < 0.05$).

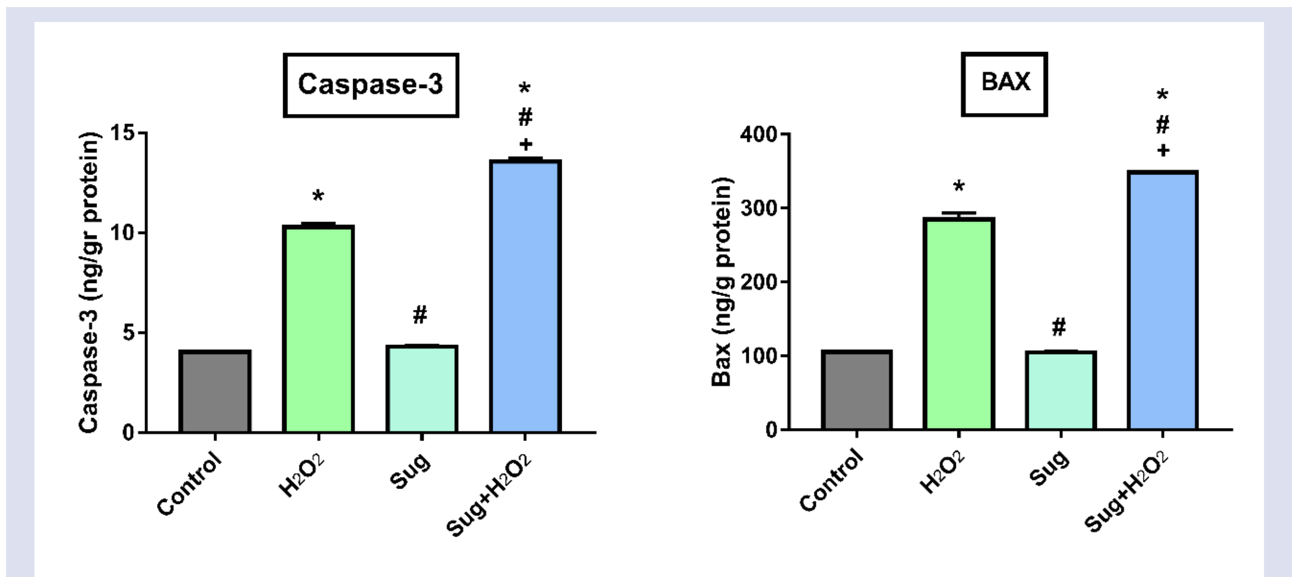


Figure 3. Effect of SUG on Caspase-3 and Bax levels in C6 cells after H2O2 -induced oxidative damage. Values are presented as mean ± SEM. *p<0.05 vs Control group; #p<0.05 vs H2O2 group; +p<0.05 vs SUG group.

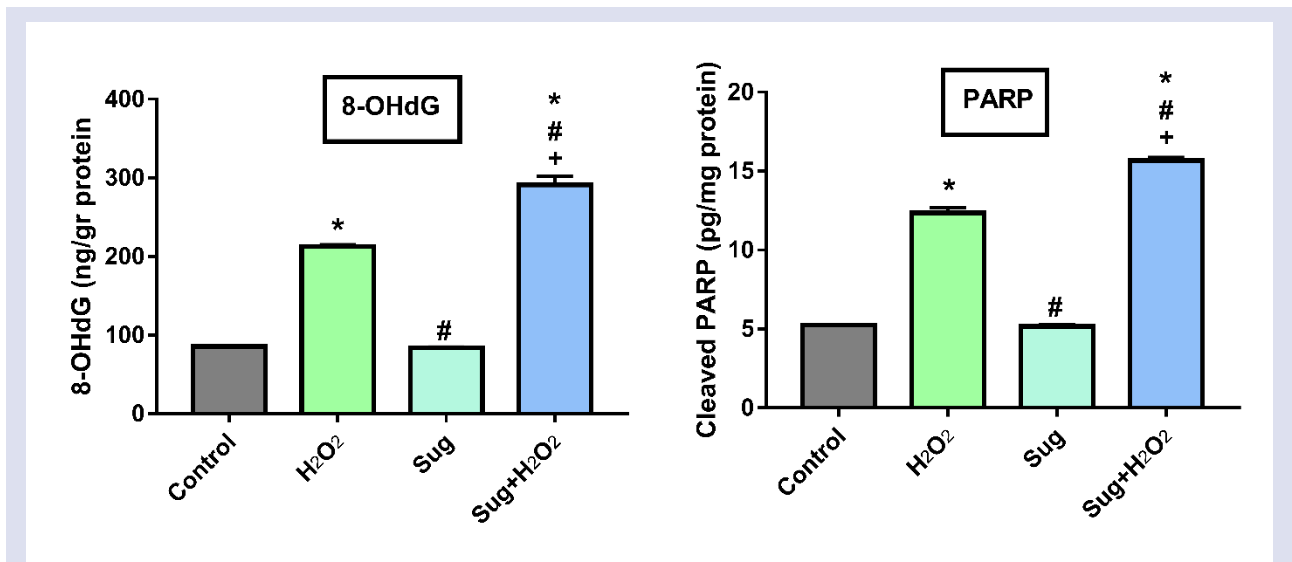


Figure 4. Effect of SUG on 8-OHdG and cleaved-PARP levels in C6 cells after H2O2 -induced oxidative damage. Values are presented as mean ± SEM. *p<0.05 vs Control group; #p<0.05 vs H2O2 group; +p<0.05 vs SUG group

Discussion

The present study, for the first time, evaluated the effect of SUG against H_2O_2-induced oxidative damage in C6 glial cells. Here, we showed that the pretreatment with SUG increased H_2O_2- induced oxidative damage in C6 cells. Moreover, SUG pretreatment also raised TOS levels and led to increased Bax, Caspase-3, 8-OHdG, and cleaved PARP levels after H_2O_2-induced oxidative damage in C6 cells.

SUG is a specifically designed agent to encapsulate the steroidal neuromuscular blocking drugs rocuronium and vecuronium. Due to the encapsulation approach, the reversal by SUG is achieved very fast and does not cause cholinergic side effects[22]. Therefore, SUG is widely used in patients under general anesthesia. However, it has been reported that SUG can lead to side effects such as pain, nausea, cough, headaches or vomiting, and to cause hypersensitivity to the anaphylactic shock from erythema[10,11,23]. SUG cannot cross a healthy and mature blood-brain barrier, but this compound can reach the brain in patients with impaired BBB function.

A previous study by Palanca et al. reported that SUG leads to cell death, mainly by apoptosis, in cultured neurons[12]. Consistently, in the current research, we found that SUG decreased C6 cell viability. However, SUG alone did not show apoptotic properties in our study; instead, it led to increase caspase-3 and Bax levels after H_2O_2-induced oxidative damage in C6 cells. The different results may be related to their different methodology and settings. The overproduction of reactive oxidant species (ROS) causes an increase in oxidative injury that damages mitochondria, cell membrane phospholipids, DNA, and protein, which also triggers cell apoptosis[24]. It has been reported that SUG-induced alteration in cholesterol homeostasis and consequent oxidative stress causes neuronal death[12]. In contrast to this study, we found that SUG alone did not change cell oxidant status, as seen at TAS and TOS levels, but the administration of SUG after H_2O_2 treatment caused an increase in oxidative stress in C6 cells. Similarly, in our study, exposure to SUG resulted in upregulated levels of 8-OHdG and cleaved-PARP after H_2O_2-induced oxidative damage in C6 cells. All in all, our results suggest that SUG alone does not trigger oxidative and apoptotic damage in neurons, but it aggravates these changes initiated by H_2O_2.

Remarkably, it has reported the SUG-induced hypersensitivity reactions[25]. This implies that the neuronal death caused by SUG may be related to the inflammatory process. To the best of our knowledge, no studies have yet been conducted on whether SUG causes inflammation in the neuron.

In conclusion, the current study shows that SUG exarbates H_2O_2-induced oxidative damage in C6 glial cells. However, further studies are needed to elucidate the mechanisms underlying SUG-induced neuronal death.

Conflicts of interest

The authors stated that did not have conflict of interests.

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