Idebenone protects against ethanol toxicity in HT-22 cells through strengthening neuroimmune response

Research Article

ABSTRACT

Idebenone, an analogue of coenzyme Q10, may function as a neuroprotective agent with its antioxidant and anti-inflammatory properties. The current report was designed to examine the beneficial effects of idebenone on ethanol-related neurotoxicity in hippocampal neuronal HT-22 cells in vitro and annotate the neuroprotective mechanism of idebenone. 75 mM ethanol was applied to the cells for 24h to develop ethanol toxicity. Then, different concentrations of idebenone (final concentration in the well to be 1, 2.5, and 5 µM) were applied to HT-22 cells for 24 h to explore the protective impact against ethanol-induced hippocampal damage. Cell viability was evaluated with MTT test. MDA, SOD, and GSH concentrations were examined to interpret oxidative damage. Moreover, the effects of idebenone on IL-1β, IL-6, and IL-23 neuroimmune-related genes expression levels were assigned by the RT-PCR analysis. In our study, 75 mM ethanol decreased neuronal cell viability by approximately 61%. All concentrations of idebenone were not toxic to neurons. In addition, idebenone increased cell viability by reducing the damage caused by alcohol. Idebenone reversed the reduction in antioxidant capacity caused by ethanol through decreasing MDA and increasing SOD and GSH levels. In addition, idebenone attenuated ethanol-induced impairment in neuroimmune and neuroinflammatory responses by reducing IL-1β, IL-6, and IL-23 mRNA expression levels. Treatment with idebenone increased antioxidant capacity and a significant improvement was achieved in neuroimmune and neuroinflammatory parameters. Possible mechanisms underlying these beneficial effects cover the down-regulation of IL-1β, IL-6, and IL-23 receptors, and antioxidant restoration of idebenone.

Keywords: Ethanol toxicity, HT-22, idebenone, neuroimmunity, oxidative damage

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INTRODUCTION

Alcohol dependence, or alcoholism, is a chronic and severe condition affecting 140 million individuals globally. In accordance with the World Health Organization, 5.3% of all global loss of life and 5.1% of the global burden of illness and injury can be based on alcohol use disorder (AshaRani et al., 2022).

Growing evidence demonstrates that ethanol exposure can lead to acute and long-term cognitive impairment including memory dysfunction, resulting in considerable disability and cost to society (Belhorma et al., 2021).

The hippocampus is one of the most-investigated brain regions which contributes to cognitive functions as well as memory, and learning (Meier et al., 2022). Substantial reports propose that one of the principal regions of influence of alcohol toxicity is the hippocampus; indeed the alcoholic population indicates neuronal forfeit and a decline of hippocampal total volume as demonstrated by magnetic resonance imaging (Meier et al., 2022; Mira et al., 2019).

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The underlying mechanisms of alcohol-induced hippocampal impairment still remain elusive. Thanks to liposoluble feature of alcohol, it can easily cross the blood-brain barrier. Acetaldehyde, a metabolized product of alcohol, is highly toxic to nerve cells as it promotes oxidative stress (Gorky and Schwaber, 2016; Peana et al., 2017). Alcohol dependence, or alcoholism, is a chronic and severe condition affecting 140 million individuals globally. In accordance with the World Health Organization, 5.3% of all global loss of life and 5.1% of the global burden of illness and injury can be based on alcohol use disorder (AshaRani et al., 2022). Growing evidence demonstrates that ethanol exposure can lead to acute and long-term cognitive impairment including memory dysfunction, resulting in considerable disability and cost to society (Belhorma et al., 2021).

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The underlying mechanisms of alcohol-induced hippocampal impairment still remain elusive. Thanks to liposoluble feature of alcohol, it can easily cross the blood-brain barrier. Acetaldehyde, a metabolized product of alcohol, is highly toxic to nerve cells as it promotes oxidative stress (Gorky and Schwaber, 2016; Peana et al., 2017). Alcohol-mediated oxidative stress includes various mechanisms such as lipid peroxidation, impairment of antioxidants inclusive of superoxide dismutase (SOD) and glutathione (GSH) levels, and DNA strand breaks (Tsermpini et al., 2022). In response to oxidative damage, glial cells including microglia, astrocytes, and also neurons intervene with neuroimmune reactions via the synthesis of neuroimmune factors, and pro-inflammatory cytokines (Crews et al., 2015). The enhanced immune activation leads to feed-forward to perpetuate inflammation (Erickson et al., 2019; Kelley and Dantzer, 2011).

Interestingly, interleukin-1 beta (IL-1β), one of the important proinflammatory cytokines, implicates in pursuing of immune responses through elevated expression from microglia and contributing to the severity of alcohol-related hippocampal neurodegeneration (Coleman et al., 2018). Mainly, IL-1β regulates the generation of IL-6 which exerts a role in immune/inflammatory responses in immunomodulation (Szelényi, 2001). IL-6 in combination with IL-23 promotes IL-17 production from memory CD4+ T cells (Nitsch et al., 2021). IL-23, a proinflammatory cytokine, release from astrocytes and infiltrating macrophages and mediating the development of neuroinflammation diseases (Lowe et al., 2018). Alcoholism changes the neuroimmune system and especially impacts the cytokines inclusive of IL-1β, IL-6, and IL-23 milieu of the brain (Kelley et al., 2011; Lowe et al., 2018). In our study, we wanted to focus on the usage of drugs currently in clinical employ for novel indications. Because the safety of these remedies is already well known and this significantly alleviates the risk of unexpected side impacts.

Idebenone, a synthetic analogue of coenzyme Q10, is a considerable endogenous antioxidant and a fundamental component of the ATP-generating mitochondrial electron transport chain and endogenous antioxidant (Suárez-Rivero et al., 2021). Although idebenone contains the identical quinone piece as CoQ, idebenone has higher solubility and bioavailability than CoQ due to its shorter hydrophobic tail (Gueven et al., 2015; Suárez-Rivero et al., 2021). Idebenone crosses the blood-brain barrier easily and provides neuroprotection in vitro and in vivo models of neuronal harm, inclusive of oxidative stress

(Jaber et al., 2020). Also, it could attenuate the expression of the proinflammatory cytokine through the overexpression of enzymes related to ameliorating lipid peroxidation such as NAD(P)H dehydrogenase quinone 1/SOD (Shastri et al., 2020).

In this context, in the current study, we aimed to explore the protective effects of idebenone in ethanol-induced hippocampal neuronal toxicity in vitro model using mouse hippocampal neuronal cell line HT-22, with a focal point on the furthermore, on the relationship of idebenone with oxidative stress and neuroimmune reactions.

MATERIALS AND METHODS

Ethanol toxicity and idebenone treatment

The mouse hippocampal neuronal HT-22 cells were kindly provided by Asst. Prof. Caner Gunaydin (Samsun University, Samsun). Cells were seeded into 96-well plates at density of 5×10^4 cells/well and left to attach overnight. As a stressor, 75 mM ethanol was added to the wells for 30 min., and ethanol toxicity was created. Then, different concentrations of idebenone (1, 2.5, and 5 µM) were applied to HT-22 cells and left for 24 hours of incubation, and the effect of the active ingredient against ethanol toxicity was evaluated. After application, it was incubated for 24 hours (5% CO_2, 95% humidity, and 37°C).

MTT tetrazolium assay concept

The MTT analysis was actualized with a commercially obtainable kit (Sigma Aldrich, USA). Succinctly, 10 µL MTT solution was put on each well and then incubated for 4 hours (5% CO_2; 37°C). Then, the medium was suspended and dimethylsulfoxide (DMSO; 100 µL) (Sigma Aldrich, USA) was suffixed to each well for dissolving formazan crystals. Cell viability (%) was quantified by optical density (OD) determined at 570 nm with the Multiskan ™ GO Microplate Spectrophotometer (Thermo Scientific, Canada, USA) (Okkay et al., 2021).

Oxidative stress parameters

Malondialdehyde (MDA), SOD, and GSH were found with a commercial kit (Elabscience, USA) with respect to producer directions as reported before Okkay et al., 2021). OD was evaluated at the 450 nm wavelength.

Measurement of relative gene expression for real-time PCR analysis

Total RNA was collected from hippocampal neuronal cells with TRIzol® reagent (Thermo Scientific, USA). Total RNA was employed for synthesizing cDNA using cDNA reverse transcription kit (Thermo Scientific, USA). The expression of IL-1β, IL-1α, and IL-23 mRNA was determined with Rotor-Gene Q (QIAGEN). Taq Man Gene Expression Master Mix kit was employed for PCR amplification and quantification. Findings were given as relative-fold in comparison to the control. We normalized IL-1β, IL-6, and IL-23 gene expressions to β-actin using the $2^{-\Delta\Delta Ct}$ method (Cicek et al., 2023). Information about the primary sequences of the genes is given in Table 1.

Table 1. Primers used for real-time PCR analysis

Genes	Forward Sequence (5′-3′)	Reverse Sequence (3′-5′)
IL-1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
IL-6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
IL-23	CATGCTAGCCTGGAACGCACAT	ACTGGCTGTTGTCCTTGAGTCC

Statistical analysis

Findings were calculated with IBM SPSS Statistics (Version 22.0, IBM Co., Chicago, IL, USA) software. All calculations were carried out by one-way analysis of variance (ANOVA) with post hoc Tukey's test. $p<0.05$ was appraised as statistically significant. Results were given as mean ± SD.

RESULTS

Effect of idebenone on ethanol-induced reduction of cell viability

As shown in Figure 1 cell viability was reduced by approximately 61% in hippocampal neuronal cells treated with 75 mM ethanol (p=0.0003). Our results also revealed that idebenone (1–5 µM) did not exhibit any toxic effect on the neurons and protect cell viability (p>0.05). As shown in Figure 1, treatment with 1, 2.5, and 5 µM idebenone for 24h induced a significant increase in cell viability by p=0.0013, p=0.0003 and p=0.0001, respectively compared with ethanol group.

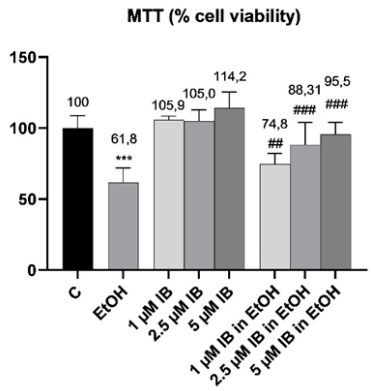

Figure 1. Cell viability in hippocampal HT-22 cell culture exposed to IB (alone) or in combination with 75 mM EtOH. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. $^{***}p<0.001$ vs. control group, $\#p<0.05$ vs. EtOH group, $^{\#\#}p<0.01$ vs. EtOH group, $^{\#\#\#}p<0.001$ vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone.

Effect of idebenone on ethanol-induced oxidative damage

As shown in Figure 2, the highest MDA level was observed in the ethanol group ($p<0.001$). Same time the increase of MDA level caused by ethanol decreased in all concentrations of idebenone treatment groups. The reduction in the MDA levels in the 1, 2.5, and 5 µM idebenone groups were statistically significant compared ethanol group (p=0.0227, p=0.0047, and p=0.0024; respectively). As shown in Figure 2, the SOD and GSH levels was the lowest in ethanol group ($p<0.001$). In addition, the decrease in SOD and GSH levels caused by ethanol increased as a result of the application of all concentrations of idebenone.

Effect of idebenone on ethanol-induced neuroimmune disorders

The mRNA expression of IL-1β (p=0.0002), IL-6 ($p<0.001$) and IL-23 (p=0.0003) were markedly up-regulated in the ethanol group compared with the control group. Transcription levels of IL-1β, IL-6 and IL-23 at all concentrations of idebenone were significantly decreased in neuronal culture compared with that of ethanol control group (Figure 3).

DISCUSSION

Alcohol is the most legal addictive drug worldwide and its excessive consumption is the third leading cause of death in the world. Excessive alcohol use is a health problem that causes brain intoxication, dementia, and cognitive disorders, and eventually to death as a result of depressive effects on the central nervous system (AshaRani et al.,2022; Belhorma et al., 2021; Meier et al., 2022; Mira et al., 2019). In addition to being a drug used for many congenital abnormalities, we think that idebenone may be a neuroprotective agent which can be improve alcohol neurotoxicity. However, there are no studies in the literature regarding the effectiveness and mechanism of action of idebenone on alcohol toxicity in hippocampal neuron culture. In this study, the effects of idebenone on neurodegeneration in the model of alcohol toxicity induced in hippocampal neuronal cells were investigated in vitro for the first time and important preclinical data on the therapeutic potential of idebenone in the treatment of alcohol toxicity were brought to the literature.

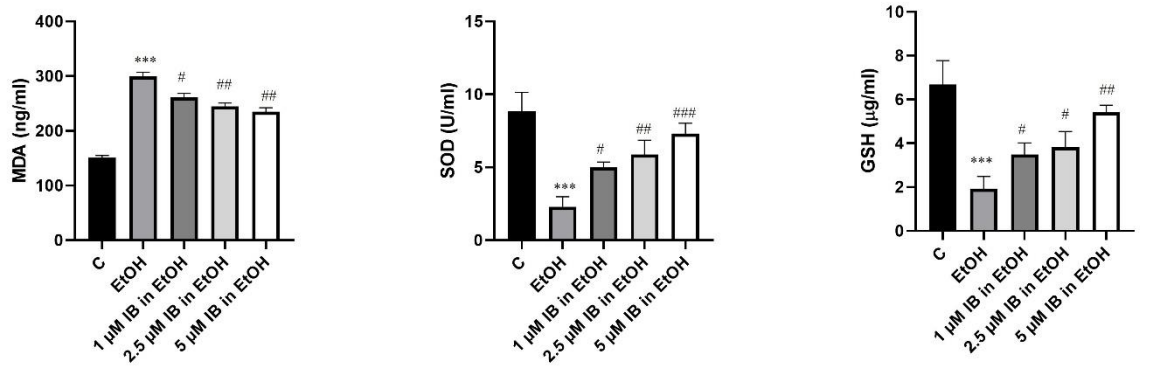

Figure 2. Effects of IB in combination with 75 mM EtOH on the oxidative stress parameters (MDA, SOD, and GSH) in hippocampal HT-22 cell culture. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. ***p<0.001 vs. control group, #p< 0.05 vs. EtOH group, ##p<0.01 vs. EtOH group, ###p<0.001 vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione.

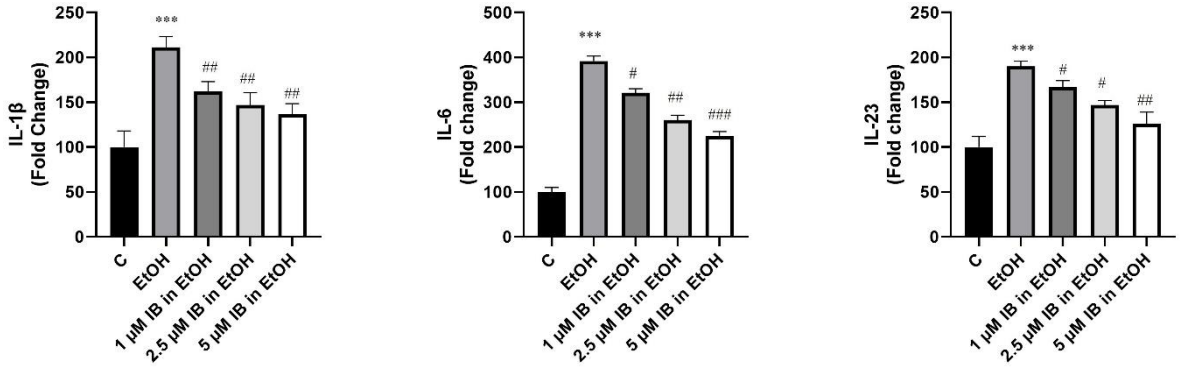

Figure 3. Effects of IB in combination with 75 mM EtOH on the neuroimmune markers (IL-1β, IL-6 and IL-23) in hippocampal HT-22 cell culture. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. ***p<0.001 vs. control group, #p<0.05 vs. EtOH group, ##p<0.01 vs. EtOH group, ###p<0.001 vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; IL-23: Interleukin 23.

The toxic effects of alcohol on neurons have been demonstrated in different preclinical models, including in vitro hippocampal, cortex cultures, etc. (Bailey et al., 2022; Bhowmick et al., 2022). Wang et al. demonstrated that hippocampal neuron injury was simulated by 200 mM ethanol in vitro and reduced cell viability by up to 70% (Wang et al., 2017). In our study, a cell model of alcohol toxicity model was first established using 75 mM ethanol, which reduced cell viability approximately 61%. Alcohol concentrations applied to hippocampal neuron cultures vary in the literature (Bailey et al., 2022; Bhowmick et al., 2022; Wang et al., 2017). However, we brought forward that these contradictions may be related to differences in cultural conditions including the continuum of cultures and the medium. Our finding demonstrated that 75 mM dose of alcohol exposure in vitro compromises the survival of cultured hippocampal neurons. Treatment with idebenone markedly protected in a in the HT-22 hippocampal neuron culture against ethanol-induced neurotoxicity and increased the cell viability. Muscolia et al. (2002) reported that liposomally entrapped idebenone reduced ethanol-induced injury of astrocytes by increasing of cellular viability. On the other hand, alcohol-related reduction of neuron viability was associated with a significant augmentation of

oxidative processes in cell cultures. Quintanilla et al. (2020) demonstrated that ethanol exposure in hippocampal neuron culture lead to enhancement of intracellular reactive oxygen species. Also, another study clearly demonstrated an increase in ethanol-induced ROS production and a decrease in GSH level and SOD and CAT activity involved in antioxidant defence (Song et al., 2015). In this report, we found that ethanol remarkably elevated MDA level and decreased SOD and GSH levels in HT-22 neuronal cells. Our results are in line with previous data demonstrating that treatment with ethanol produces oxidative stress and decreased efficiency of the cellular antioxidant mechanisms. Therefore, idebenone reversed neuronal damage in the ethanol-induced model by decreasing hippocampal neuronal oxidative stress. It is probably neuroprotective characteristics of idebenone by virtue of its antioxidant activity because it has been declared that antioxidants including α-tocopherol prevented the neurotoxicity (Lee et al., 2022).

It is renowned that oxidative stress can induce neuroimmune response and neuroinflammation (Dukay et al., 2021; Simpson and Oliver, 2020). Several studies have reported increases in the expression of genes encoding components of the IL-1β signaling pathways in neuron with a genetic predisposition to alcohol consumption (Patel et al., 2019; Varodayan et al., 2023). Alfonso-Loeches et al. (2016) findings show that ethanol triggers NLRP3 inflammasome activation in microglial cells and cultured microglia to cause the release of IL-1β which could amplify alcohol-induced neuroinflammation. Interestingly, the impaired neuroimmune response by chronic ethanol exposure is also associated with an elevation of IL-6 levels (Gano et al., 2017). It was also demonstrated that elevation of IL-6 in the hippocampus after ethanol intoxication is associated with the neuroimmune effects of ethanol (Gano et al., 2019). Our finding indicating that ethanol stimulated IL-6 expression in hippocampal cultures, is directly in line with this report. IL-23, a messenger of the immune system, is primarily secreted by microglia and infiltrating macrophages under inflammatory conditions (Nitsch et al., 2021). Also Lowe et al. (2018) demonstrated that alcohol treatment significantly increased the expression of IL-6 and IL-23 pro-inflammatory cytokine in the brain which was in line with our results. The data we obtained are consistent with the effects of ethanol: influences the neuroimmune system, specifically eliciting an increase in IL-1β, IL-6 and IL-23 proinflammatory cytokines. However, idebenone reversed the increased IL-1β, IL-6 and IL-23 induced by ethanol. These results indicate that restoration of neuroimmune response is a probable neuroprotective action of idebenone following ethanol exposure. The study of the effects of IL-1β, IL-6 and IL-23 on neuroimmune and neuroinflammation on the ethanol toxicity of idebenone for the first time in our study constitutes the specificity of our study.

CONCLUSION

Our results indicated that exposure of hippocampal rat neuron culture to ethanol caused functional changes which are related to oxidative stress, neuroimmune response and neuroinflammation. These results are also confirmed by the evidence that the protective effects of idebenone on ethanol-induced toxicity might be associated with antioxidative and anti-inflammatory effects as well as the modulation of neuroimmune response via IL-1β, IL-6 and IL-23 signaling pathway. This may demonstrate a new and potentially useful approach to idebenone in the treatment of ethanol-induced neurodegenerative disorders.

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Not applicable

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

Conflict of interest: There is no conflict of interest between the authors.

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