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Dapsone Protects Against Lithium-Pilocarpine-Induced Status Epilepticus in Rats through Targeting Tumor Necrosis Factor-α and Nitrergic Pathway

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Background and Purpose: Status epilepticus (SE) results in permanent neuronal brain damage in the central nervous system. One of the complex etiologies underlying SE pathogenesis is neuroinflammation. Dapsone has been recently considered as a potential neuroprotective agent in neuroinflammatory conditions. Therefore, the present study aims to investigate effects of dapsone on lithium-pilocarpine-induced SE in rats and assess whether tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) pathway participate in this effect.

Methods: SE was established by injecting lithium chloride (127 mg/kg, intraperitoneally [i.p.]) and pilocarpine (60 mg/kg, i.p.). The animals received pre-treatment dapsone (2, 5, 10, and 20 mg/kg, oral gavage) and post-treatment dapsone (10 mg/kg). Subsequently, seizure score and mortality rate were documented. To assess the underlying signaling pathway, L-Nω-Nitro-L-arginine methyl ester hydrochloride (a non-specific NO synthase [NOS] inhibitor), 7-nitroindazole (a specific neuronal NOS inhibitor), and aminoguanidine (a specific inducible NOS inhibitor) were administered 15 minutes before dapsone (10 mg/kg) pre- or post-treatment. Hippocampal tissue TNF-α and NO concentrations were quantified using the enzyme-linked immunosorbent assay method.

Results: Dapsone (10 mg/kg) pre-and post-treatment significantly attenuated the increased seizure score and mortality rate due to lithium-pilocarpine-induced SE. The development of SE in animals was associated with higher TNF-α and NO metabolites levels, which notably decreased in the dapsone-treated rats. Moreover, co-administration of NOS inhibitors with dapsone markedly reversed the anti-epileptic effects of dapsone and caused an escalation in TNF-α level but a significant reduction in NO concentration level.

Conclusions: It seems that dapsone may exert an anti-epileptic effect on lithium-pilocarpine-induced SE through TNF-α inhibition and modulation of the nitrergic pathway. **(2022;12:39-47)**

Key words: Dapsone, Status epilepticus, Nitric oxide, Tumor necrosis factor-alpha

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Introduction

Status epilepticus (SE) is a life-threatening condition that occurs as episodes of recurrent or persistent seizure activity and unconsciousness.[1] These seizures continue unabated and cause permanent neuronal injury in the central nervous system (CNS), and even death.[2,3] According to previous clinical and experimental studies, multiple molecular pathways play an essential role in the pathogenesis of SE, including overexpression of pro-inflammatory factors and nitric oxide (NO).[4,5] In addition, pro-inflammatory cytokines and SE exacerbate each other by creating an insidious cycle of neurotoxic damage, and there is a strong relationship between SE and increasing tumor necrosis factor-alpha (TNF-α) in the brain.[6] Therefore, accumulating evidence has indicated the beneficial use of anti-inflammatory drugs

for epileptic disorders and SE.[7]

There are several studies that have shown that the well-known bacteriostatic agent dapsone (4, 40 diamino-diphenyl sulfone, DDS) has neuroprotective and anti-inflammatory properties.[8,9] Accumulating evidence indicate the beneficial effects of dapsone on seizure activities, including its anti-convulsive effects against the amygdala-kindled seizures in cats and rats[10] and neutralization of the neuronal damages induced by quinolinic acid in rats.[11] Furthermore, dapsone may have anti-inflammatory effects via suppressing the pro-inflammatory interleukin (IL)-8 levels in seizures in clinical studies.[12] In addition, dapsone inhibits TNF-α overproduction and cell death in lipopolysaccharide-activated bone marrow cells.[13]

NO is an essential messenger in the central and peripheral nervous systems.[14] NO is generated from L-arginine as a result of three isoforms of NO synthesize (NOS) activities containing neuronal NOS (nNOS) that are found in neuronal tissues, endothelial NOS (eNOS) that express in endothelial tissues, and inducible NOS (iNOS) that secrete by inflammatory cells.[15-17] Previous studies revealed that SE activated glial cells and intensified pro-inflammatory agents including TNF-α, and NO production, which are responsible for cell death and dysfunction in neuroinflammatory diseases.[18-20] Therefore, preventing the activation of TNF-α and NO pathways may control some neurological and peripheral effects.[21,22]

The aim of the present study was to evaluate the effects of dapsone on lithium-pilocarpine-induced SE in male rats and to determine the probable role of the TNF-α and NO signaling pathway in this effect of dapsone.

Methods

Chemicals and drug administration

Chemical used in our study were as follows: 1) dapsone in the treatment groups; 2) pilocarpine, lithium chloride, and scopolamine methyl bromide in order to induce the SE; and 3) L-Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), aminoguanidine (AG), and 7-nitroindazole (7-NI) to evaluate the probable role of NO. Dapsone was a gift from Gilaranco pharmaceuticals. Other chemicals were obtained from Sigma-Aldrich corporation. Dapsone was liquefied in 4% dimethyl sulfoxide and was administered directly into the stomach via gastric gavage. The other solutions were administered intraperitoneally (i.p.) as freshly prepared by dissolving in physiological saline except for 7-NI that dissolved in tween 80. The chemical dosage, route of injection, and administration time were found based on our previous hypothesis, pharmacokinetic consideration, and pilot studies.[8,23]

Animal and housing

All adult male Wistar rats (210-260 g, 2 months old) were provided from Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences. The rats were accustomed to a controlled room temperature setting (12-hour light/dark cycle, 23°C, and humidity of 60%). The male rats were supplied with unrestricted

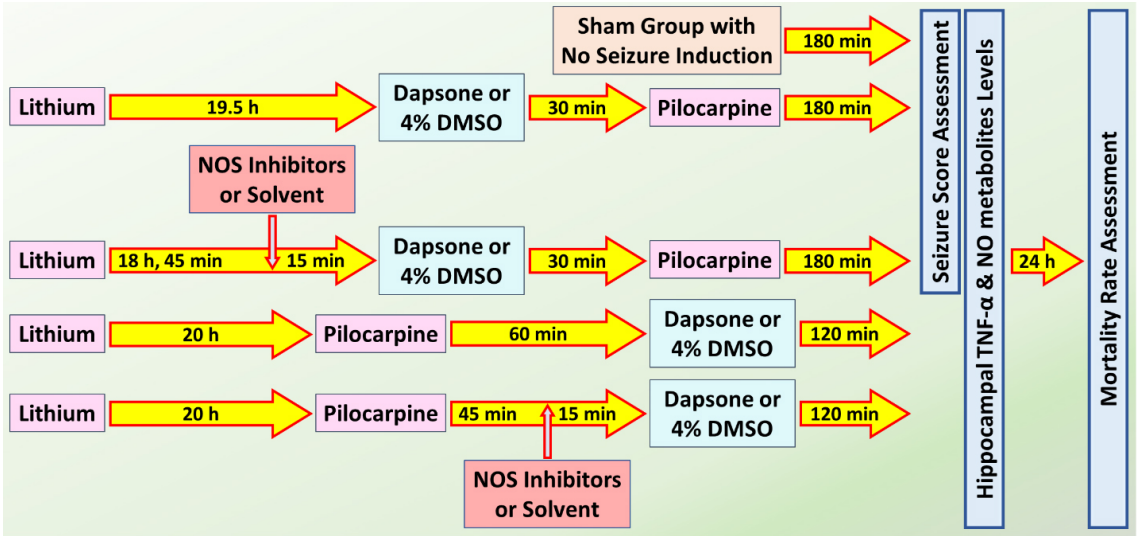

Figure 1. Schematic representation of drug administration and its timeline prior or after the lithium-pilocarpine model of status epilepticus in rats in different experimental groups. NOS, nitric oxide synthase; DMSO, dimethyl sulfoxide; TNF-α, tumor necrosis factor-alpha; NO, nitric oxide.

access to food and bottles containing tap water. We used 160 rats divided randomly into main seven groups and required subgroups, as follows: 1) the sham group, 2) the SE-control group, 3) the SE + pre-treatment dapsone (2, 5, 10, and 20 mg/kg) groups, 4) the SE + post-treatment dapsone group, 5) the SE + NOS inhibitors per se groups (L-NAME, 7-NI, AG), 6) the SE + pre-treatment dapsone + NOS inhibitors groups, and 7) the SE + post-treatment dapsone + NOS inhibitors groups. The lowest efficacious dose of dapsone (10 mg/kg) in group 3 was applied in other experimental groups. Each group consisted of 9-10 rats, and each animal was used only once during the experiment.

Induction of SE and drug treatments

Animals received an injection of lithium (127 mg/kg, i.p.) and pilocarpine (60 mg/kg, i.p.) to induce SE. Pilocarpine was administered 20 hours after lithium injection. Thirty minutes preceding pilocarpine injection, scopolamine (2 mg/kg, i.p.) was administered to mitigate the peripheral cholinergic detrimental effects. Afterward, the symptoms of SE development in the pre-treatment experimental groups were scored based on 5-points racine scale by a blinded appraiser to the experimental procedures (Fig. 1).[24] Animals in the sham group received the vehicle without the SE induction. In the SE-control group, the vehicle was used as a treatment. To evaluate the effect of dapsone (10 mg/kg, oral gavage), we used dapsone as a pre-treatment (half an hour before inducing SE) and post-treatment (60 minutes after SE induction) intervention.[25,26] To evaluate the regulatory effect of NO on seizure activity, L-NAME (10 mg/kg), 7-NI (30 mg/kg), or AG (100 mg/kg) were administered 15 minutes prior to dapsone/vehicle gavage in both pre-and post-treatment groups.

Rats in the pre-treatment experimental groups were monitored for 180 minutes after receiving pilocarpine, and the seizure score was recorded. Six rats were kept at their home cage in each experimental group to document the mortality rate 24 hours after SE induction (Fig. 1). The other three to four rats in each group were sacrificed under general anesthesia (ketamine 87 mg/kg and xylazine 13 mg/kg). The rat's hippocampi samples were immediately dissected on an ice-cold plate and reserved at -80°C temperature for the laboratory assessment (Fig. 1).

Biochemical assays

Measurement of TNF-α level in the hippocampus: to measure the TNF-α levels of hippocampi specimens, an enzyme-linked immunosorbent assay (ELISA) kit (RAB0479) was employed. An ELISA detector measured the absorbance value at 540 nm. The TNF-α concentration was expressed as pg/mg-p.

Measurement of NO metabolites in the hippocampus: the NO metabolites were measured according to the quantitative sandwich enzyme immunoassay method using an ELISA kit (ab65327). The absorbance was determined at 450 nm using an Elisa detector. The results were described based on μmol/gram-protein.

Statistical analysis

GraphPad Prism ver. 9.2.0 software (GraphPad Software, San Diego, CA, USA) was employed for statistical analysis. The Kruskal Wallis test was used to compare differences in seizure score among the experimental groups. Differences in NO and TNF-α concentration were assessed by one-way analysis of variance (ANOVA). Mortality rate data were analyzed by chi-square analysis with Fisher's exact correction. All values are present as mean±standard deviation (SD). The statistical significance level was set at p-values less than 0.05.

Ethics approval statement

All procedures were performed following the ARRIVE guidelines for the care and use of laboratory animal ethics committee of Tehran University of Medical Sciences (ethics code: IR.TUMS.MEDICINE.REC. 1398.839) and approved by the National Institutes of Health (NIH publication NO. 85-23; revised 1985).

Results

Seizure score and mortality rate in lithium-pilocarpine-induced SE

As the SE was induced, the pre-and post-treatment effect of dapsone was evaluated. Racine scoring scale was applied to assess the seizure score in the epileptic animals of pre-treatment experimental groups. To find the impact of dapsone on mortality rate, we observed the death rate of animals 24 hours after the SE induction.

Dapsone pre-and post-treatment effect

After the induction of SE, the seizure score increased in all animals that received lithium-pilocarpine and vehicle treatment as opposed to the animals in the sham group (SE-control, $p<0.001$). Pre-treatment with four diverse doses of dapsone (2, 5, 10, and 20 mg/kg, oral gavage) revealed a dose-related alteration in the fluctuation of the

seizure score. Dapsone (10 and 20 mg/kg) significantly reduced the seizure score compared with the SE-control group (p<0.01 and p<0.05, respectively) (Fig. 2).

NO signaling pathway effect

To find the potential regulatory function of NO on the dapsone-induced neuroprotection, we administered NOS inhibitors before dapsone pre-treatment. A non-effective dose of L-NAME (10 mg/kg, i.p.) was administered as a non-specific NOS inhibitor. Although seizure score remained unaffected by L-NAME (10 mg/kg, i.p.) alone, co-injection of L-NAME (10 mg/kg, i.p.) with dapsone (10 mg/kg, oral gavage) completely inhibited the protective effect of dapsone (10 mg/kg, oral gavage) on seizure score (p<0.01) (Fig. 3A).

A non-effective dose of 7-NI (30 mg/kg, i.p.) was injected as a nNOS. In solely administering 7-NI (30 mg/kg, i.p.), the seizure score remained unchanged. However, concomitant injection of 7-NI (30 mg/kg, i.p.) and dapsone (10 mg/kg, oral gavage) remarkably withdrawn the protective effect of dapsone (10 mg/kg, oral gavage) on the seizure score (p<0.05) (Fig. 3B).

A non-effective dose of AG (100 mg/kg, i.p.) was applied as an iNOS inhibitor. Seizure score remained unmoved by solely application of AG (100 mg/kg, i.p.), while co-injection of AG (100 mg/kg, i.p.) with dapsone (10 mg/kg, oral gavage) significantly neutralized the protective effect of dapsone (10 mg/kg, oral gavage) on the seizure score (p<0.01) (Fig. 3C).

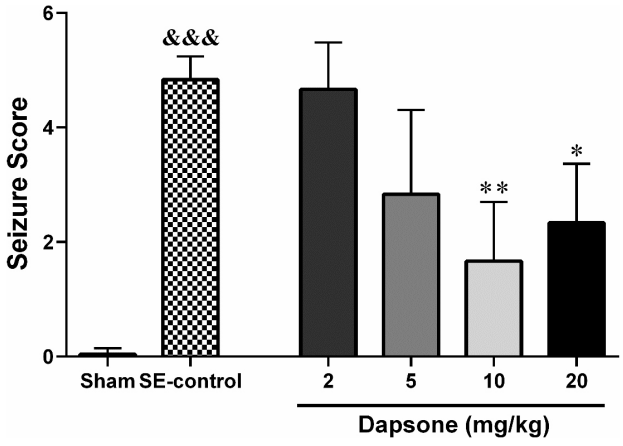

Figure 2. Effects of lithium-pilocarpine-induced status epilepticus (SE-control) on seizure score compared with the sham group. Effects of four diverse doses of dapsone (2, 5, 10, and 20 mg/kg, oral gavage) on seizure score in the lithium-pilocarpine-induced status epilepticus in rats. $^{\&\&\&}p$<0.001 as opposed to the sham group. *p<0.05 and **p<0.01 compared with the SE-control group. Each group consisted of six rats.

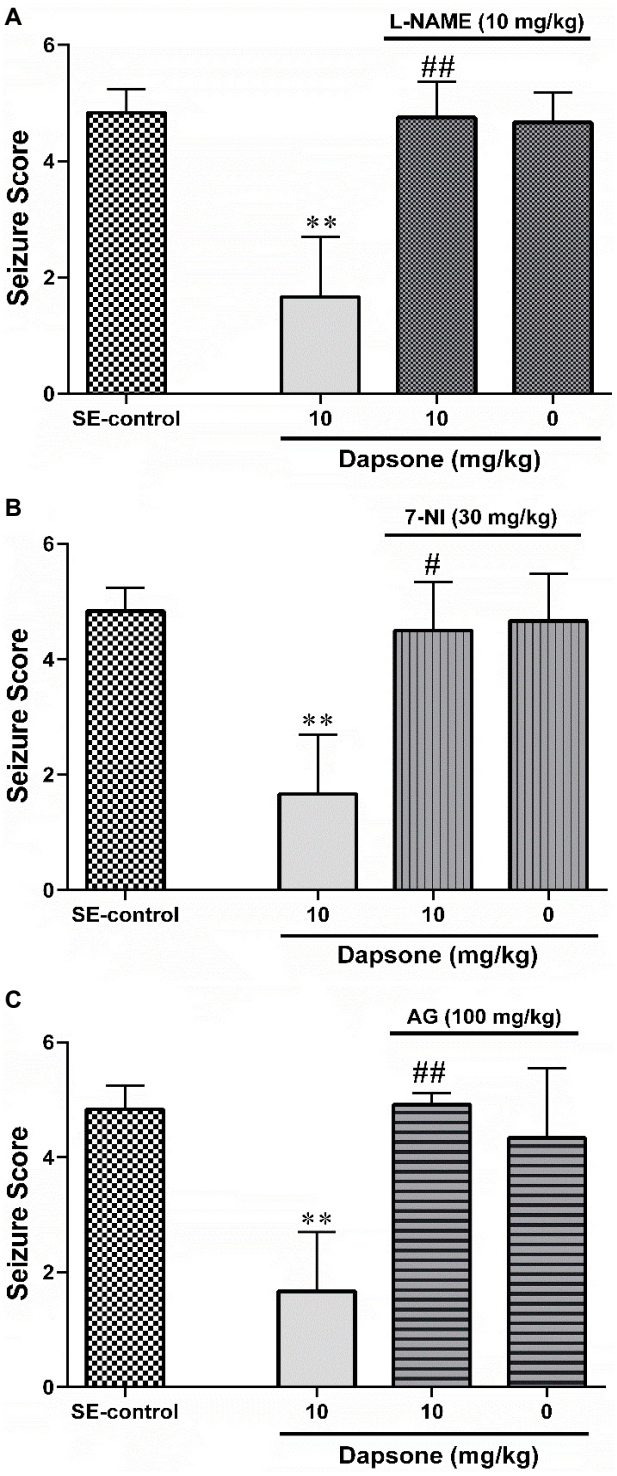

Figure 3. Effect of pre-treatment with (A) L-Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME; 10 mg/kg, intraperitoneally [i.p]), (B) 7-nitroindazole (7-NI; 30 mg/kg, i.p), or (C) aminoguanidine (AG; 100 mg/kg, i.p) before an effective dose of dapsone (10 mg/kg, oral gavage) on seizure score in the lithium-pilocarpine-induced status epilepticus (SE) in rat. **p<0.01 as opposed to the SE-control group. $^{\#}p$<0.05 and $^{\#\#}p$<0.01 compared with to the dapsone group. Each group consisted of six rats.

Mortality rate

Table 1 shows the mortality rate 24 hours after the SE induction. In the sham-treated animals, the mortality rate was zero (0%). In the SE-control group, the induction of SE after 24 hours resulted in the death of all animals (mortality rate, 100%) ($p<0.001$). In the dapsone pre-treatment animals, the mortality rate 24 hours post SE induction decreased to 16% compared to the SE-control group ($p<0.001$). As displayed in Table 1, co-treatment of NOS inhibitors; L-NAME (10 mg/kg), 7-NI (30 mg/kg), or AG (100 mg/kg) with dapsone (10 mg/kg) notably enhanced the death rate compared with dapsone alone (10 mg/kg) group ($p<0.01$, $p<0.05$, and $p<0.05$, respectively). In the dapsone post-treatment animals, the mortality rate after SE induction declined to 0% in contrast to the SE-control group ($p<0.001$). Resembling dapsone pre-treatment groups, the death rate in rats that treated with any of the NOS inhibitors (L-NAME [10 mg/kg], 7-NI [30 mg/kg], or AG [100 mg/kg]) plus dapsone (10 mg/kg) was significantly higher than the dapsone post-treatment animals ($p<0.001$, $p<0.05$, and $p<0.01$, respectively).

Biochemical findings in lithium-pilocarpine-induced SE

The tissue levels of TNF-α and NO metabolites were assessed in the hippocampal samples of the experimental groups.

Changes in TNF-α level following SE

As shown in Fig. 4A, the hippocampal TNF-α levels were significantly ($p<0.05$) higher in the SE-control rats than the sham group without SE induction. However, either pre- or post-treatment with dapsone (10 mg/kg) markedly ($p<0.01$) decreased TNF-α levels compared with the SE-control group ($F_{3,8}=15.37$, $p=0.0011$). L-NAME treatment alone at 10 mg/kg did not significantly ($p>0.05$) alter the hippocampal TNF-α levels compared with the SE-control group. However, L-NAME (10 mg/kg) pre-treatment concurrent with either pre- or post-treatment dapsone (10 mg/kg) reversed the ameliorating effects of dapsone alone (10 mg/kg) on the TNF-α levels ($F_{6,14}=21.28$, $p<0.0001$). 7-NI treatment alone at 30 mg/kg had no significant ($p>0.05$) effects on the TNF-α levels in the hippocampal tissues from SE rats compared to the SE-control group. However, concurrent administration of 7-NI (30 mg/kg) with either pre- or post-treatment dapsone (10 mg/kg) significantly ($p<0.05$) reversed the effects of dapsone alone on the TNF-α levels ($F_{6,14}=8.197$, $p=0.0006$). AG treatment alone at 100 mg/kg did not significantly ($p>0.05$) change the hippocampal TNF-α levels in SE rats compared to the SE-control group. However, concurrent administration of AG (100 mg/kg) with either pre- or post-treatment dapsone (10 mg/kg) significantly ($p<0.001$) reversed the effects of AG alone on TNF-α levels ($F_{6,14}=32.04$, $p<0.0001$).

Changes in NO metabolites level following SE

As shown in Fig. 4B, the hippocampal NO metabolites level in the SE-control group was significantly ($p<0.001$) elevated compared with the sham-treated rats without SE induction. However, either pre- or post-treatment with dapsone (10 mg/kg) markedly ($p<0.01$) decreased NO levels compared with the SE-control group ($F_{3,8}=18.77$, $p=0.0006$). L-NAME treatment alone at 10 mg/kg also significantly ($p<0.001$) decreased NO metabolites in the hippocampal samples compared with the SE-control group. Notably,

Table 1. Mortality rate in the different experimental groups after 24 hours

Group	Dapsone pre-treatment	Dapsone post-treatment
Sham	0.0% (0/6)	0.0% (0/6)
SE-control	100.0% (6/6)*	100.0% (6/6)*
Dapsone (10 mg/kg)	16.0% (1/6)†	0.0% (0/6)†
Dapsone (10 mg/kg) + L-NAME (10 mg/kg)	83.0% (5/6)§	100.0% (6/6)**
Dapsone (10 mg/kg) + 7-NI (30 mg/kg)	66.0% (4/6)‡	66.0% (4/6)‖
Dapsone (10 mg/kg) + AG (100 mg/kg)	66.0% (4/6)‡	83.0% (5/6)¶

SE, status epilepticus; L-NAME, L-Nω-Nitro-L-arginine methyl ester hydrochloride; 7-NI, 7-nitroindazole; AG, aminoguanidine.
*$p<0.001$ compared with the sham group.
†$p<0.001$ compared with the SE-control group.
‡$p<0.05$.
§$p<0.01$ compared with the pre-treatment dapsone group.
‖$p<0.05$.
¶$p<0.01$.
**$p<0.001$ compared with the post-treatment dapsone group.

L-NAME (10 mg/kg) pre-treatment concurrent with either pre- or post-treatment dapsone (10 mg/kg) resulted in a further significant decrease in NO levels compared to the L-NAME or dapsone alone groups ($F_{6,14}$=31.77, p<0.0001). 7-NI treatment alone at 30 mg/kg had no significant (p>0.05) effects on the NO metabolites level in the hippocampi samples of SE rats compared to the SE-control group. However, concurrent administration of 7-NI (30 mg/kg) with either pre- or post-treatment dapsone (10 mg/kg) significantly decreased NO levels compared to the 7-NI (p<0.01) or dapsone (p<0.05) alone groups ($F_{6,14}$=17.85, p<0.0001). AG treatment alone at 100 mg/kg did not significantly (p>0.05) alter the NO metabolites level in the hippocampi samples of SE rats compared to the SE-control group. However, concurrent administration of AG (100 mg/kg) with either pre- or post-treatment dapsone (10 mg/kg) significantly decreased NO levels compared to the AG (p<0.001) or dapsone (p<0.01 and p<0.01 for pre- and post-treatment, respectively) alone groups ($F_{6,14}$=21.10, p<0.0001).

Discussion

In this study, we presented the potency of dapsone in attenuating the seizure score and mortality rate in epileptic animals. Dapsone revealed the anti-convulsive properties in either pre- or post-treatment of SE. Although this effect seemed to be dose dependent, dapsone at 20 mg/kg had slightly less effect than 10 mg/kg. We did not use a dose higher than 20 mg/kg in our current study to see whether there are any bimodal effects on seizure scores, as we tried to avoid potential adverse or toxic effects that may occur with higher dose of the drug. Administration of non-selective (L-NAME) and selective (nNOS and iNOS) NOS inhibitors prior to dapsone reversed the anti-convulsant effect of dapsone and led to increased seizure score and mortality rate in epileptic animals. However, exposing the animals to NOS inhibitors alone did not change the seizure score and mortality rate significantly.

The biochemical assessment showed that dapsone administration prevents the overproduction of TNF-α and NO in the hippocampi samples of SE animals. Furthermore, injection of L-NAME, 7-NI, and AG before dapsone gavage withdrawn the treatment effect. Altogether, we suggest that the TNF-α and NO signaling transduction are the key players in the inflammation process of lithium-pilocarpine-induced SE, which successfully ameliorates through dapsone administration.

SE is an emergency condition known as seizures with altered electrical neuronal activity, disturbing the balance between inhibitory and excitatory signal transmission in the brain.[27,28] For instance, alternation in the cholinergic neurotransmitters eventually results in

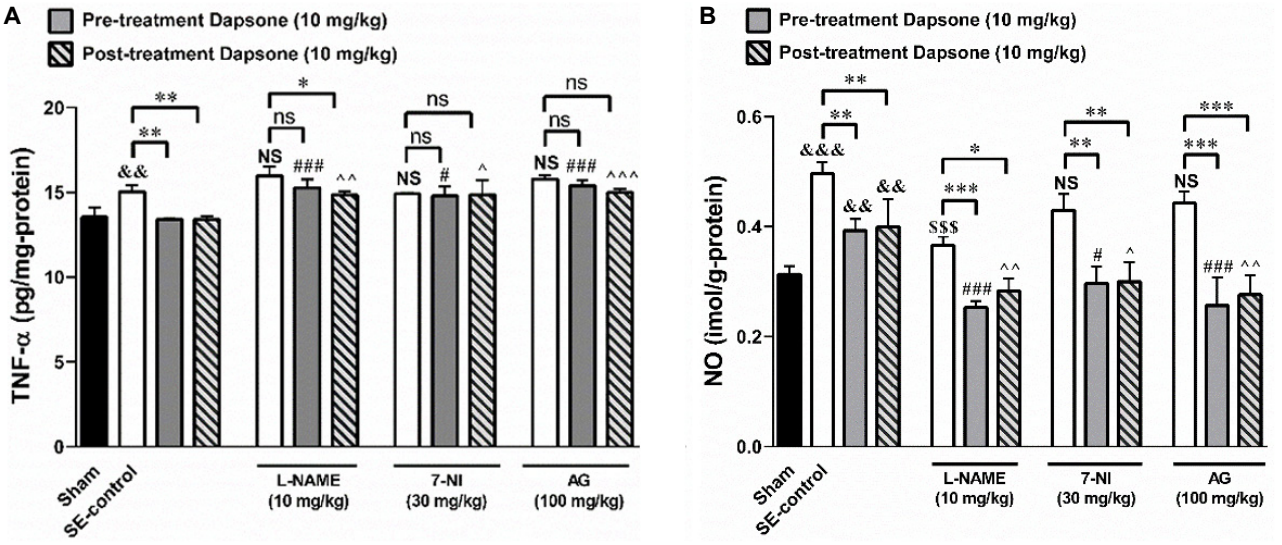

Figure 4. Concentration of (A) tumor necrosis factor-alpha (TNF-α) and (B) nitric oxide (NO metabolites in the hippocampal samples from lithium-pilocarpine-induced status epilepticus (SE) rats. $^{\&\&}p$<0.01 and $^{\&\&\&}p$<0.001 compared with the sham group without SE induction. Non-significant (NS) as well as *p<0.05 and **p<0.01 compared with the corresponding SE group without dapsone treatment. NS (p>0.05) and $^{\$\$\$}p$<0.001 compared with the SE-control group without dapsone treatment. $^{\#}p$<0.05 and $^{\#\#\#}p$<0.001 compared with the pre-treatment dapsone group. $^{\wedge}p$<0.05, $^{\wedge\wedge}p$<0.01, and $^{\wedge\wedge\wedge}p$<0.001 compared with the post-treatment dapsone group. Each group consists of three animals. L-NAME, L-Nω-Nitro-L-arginine methyl ester hydrochloride; 7-NI, 7-nitroindazole; AG, aminoguanidine.

temporal lobe epilepsy (TLE). Induction of SE by lithium-pilocarpine (cholinergic antagonist) is compatible with clinical and neuropathological features reported in human TLE. Utilizing this model in animals provides beneficial outcomes in developing novel treatments against intractable seizures.[29-31] Hence, we used lithium-pilocarpine as a validated model of epilepsy to induce SE in rats. The initiation of SE causes overexpression of pro-inflammatory agents, including TNF-α, IL-1β, and IL-6 in patients suffering from epilepsy which ultimately results in the systemic inflammatory and anti-inflammatory response.[32,33] In other words, SE triggers inflammation-like reactions that also contribute to the deterioration of the SE condition.[5]

Dapsone is an anti-inflammatory medication commonly prescribed as a treatment of leprosy.[34] In previous studies, dapsone has been shown to possess neuroprotective impact via anti-oxidant and anti-inflammatory effects in depression and anxiety-like disorders.[35] Accumulating evidence has confirmed that single-dose administration of dapsone inhibits the inflammatory pathways in different animal models such as testicular torsion/detorsion, acetic-acid induced inflammatory bowel disease, and cuprizone-induced demyelination.[8,36,37] Notably, there is evidence that dapsone improved the anti-convulsant activity of diazepam in the kainic acid-induced SE in rats;[38] however, the anti-epileptic effect of dapsone on pro-inflammatory cytokines including TNF-α and the NO signaling pathway in the lithium pilocarpine-induced SE is still unknown.

We observed that dapsone could effectively alleviate the enhanced level of NO and TNF-α during SE development. The inflammatory response due to SE is also dependent on inflammatory modulators, including TNF-α and NO.[39] Inflammation orchestrates the infiltrating immune cells, including microglia and astrocyte cells, ultimately resulting in the elevation of TNF-α and IL-6. On the other hand, TNF-α via the induction of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) promotes the activation of iNOS.[40] Consequently, the iNOS expression increases and causes NO overproduction.[41] Likewise, NO operates as a neuromodulator agent in the CNS and triggers the release of inflammatory products.[42] Given these points, this never-ending loop circuit of inflammatory markers and NO will not stop independently. Thus, employing novel treatments like dapsone that can decrease both TNF-α and NO production seems to generate neuroprotection against epileptic tolerance.

In conclusion, pre-and post-treatment with dapsone exerts anti-convulsive effects against lithium-pilocarpine-induced SE in rats through modulating the TNF-α and NO signaling transduction. The observed anti-epileptic effect of dapsone is reversed by the NOS inhibitors (L-NAME, 7-NI, and AG).

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgment

All procedures were performed following the guidelines for the care and use of laboratory animal ethics committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.MEDICINE.REC.1398.839) and approved by the National Institutes of Health (NIH publication NO. 85-23; revised 1985). These data were not subjected to clinical trial or involving human studies. It is confirmed that all methods have been performed following the relevant guidelines and regulations. Also, it is confirmed that the study was conducted in compliance with the ARRIVE guidelines.

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