*Turk J Pharm Sci 13(1), 103-114, 2016*

**Original article**

# Effect of Mefenamic Acid on Some of The Base Excision Repair Enzymes Against D-Serine-Induced Neurotoxicity

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N-methyl-D-aspartate receptor (NMDAR) overactivation leads to free radical production, protein degradation, lipid peroxidation and DNA damage. Recently, nonsteroidal antiinflammatory drugs (NSAIDs) are suggested to be good candidates for the treatment of neurological insults. In this study, we aimed to evaluate the effect of mefenamic acid on 8-OHdG levels, the expression of poly(ADP ribose) polymerase-1 (PARP-1) and base excision repair (BER) enzymes; 8-oxoguanine DNA glycosylase 1 (OGG1), apurinic/apyrimidinic endonuclease 1 (APE1) against D-serine. Adult Sprague-Dawley rats were divided into four groups: (i) the control (*n*=6); (ii) D-serine (*n*=6); (iii) Mefenamic acid (*n*=6); (iv) D-serine+Mefenamic acid (*n*=6). Rats were decapitated 6 hours after the injections. The mRNA and protein expression levels were determined by real-time PCR and western blot techniques, respectively. D-serine increased APE1 mRNA, PARP-1 mRNA and 8-OHdG levels. APE1 and PARP-1 genes were significantly upregulated by mefenamic acid. Protein expression profiles were also consistent with mRNA levels. However neither mRNA nor protein levels of OGG1 were affected by D-serine or mefenamic acid. Our results suggest that NMDA/D-serine signaling triggers DNA repair mechanisms and oxidative DNA damage simultaneously. We may conclude that mefenamic acid have a potential neuroprotective effect and assist to repair NMDAR-mediated DNA damage via modulating DNA repair mechanisms.

**Key words:** Base excision repair, D-serine, DNA damage, Mefenamic acid

## D-serin ile İndüklenen Nörotoksisitede Mefenamik Asit'in Baz Eksizyon Tamir Enzimleri Üzerine Etkisi

N-metil-D-aspartat reseptör (NMDAR) aktivasyonu serbest radikal üretimi, protein degredasyonu, lipid peroksidasyonu ve DNA hasarına yol açar. Son yıllarda, nonsteroidal antiinflamatuar ilaçlar (NSAID) nörolojik bozuklukların tedavisinde iyi bir aday olarak gösterilmektedir. Bu çalışmadaki amacımız; baz eksizyon tamir enzimleri [8-oksoguanin DNA glikozilaz 1 (OGG1) ve apürinik/apirimidinik endonükleaz 1 (APE1)], poli ADP riboz polimeraz-1 (PARP-1) ve 8OHdG düzeyleri üzerinden D-serine karşı mefenamik asitin etkisini değerlendirmektir. Yetişkin Sprague-Dawley sıçanlar 4 gruba ayrıldı: (i) kontrol *(n*=6); (ii) D-serin (*n*=6); (iii) Mefenamik asit *(n*=6); (iv) D-serin+Mefenamik asit (*n*=6). Sıçanlar enjeksiyondan 6 saat sonra dekapite edildi. mRNA ve protein ekspresyon seviyeleri sırasıyla gerçek zamanlı PZR ve western blot teknikleriyle belirlendi. D-serin uygulaması sonucunda APE1 mRNA, PARP-1 mRNA ve 8-OHdG seviyelerinin arttığı saptanmıştır. APE1 ve PARP-1 genleri mefenamik asit uygulamasıyla anlamlı şekilde artmıştır. Bu enzimlerin protein ekspresyon düzeyleri de ölçülen mRNA düzeyleriyle tutarlıdır. Diğer taraftan, OGG1 mRNA ve protein düzeyleri mefenamik asit veya D-serin uygulamasından etkilenmemiştir. Elde edilen sonuçlar, NMDA/D-serin sinyalizasyonunun DNA tamir mekanizmaları ile oksidatif DNA hasarını simültane olarak tetiklediği düşüncesini desteklemektedir. Bu sonuçlara dayanarak, mefenamik asitin potansiyel nöroprotektif etkiye sahip olduğu ve DNA tamir mekanizmalarını düzenleme yoluyla NMDAR bağımlı DNA hasarını tamir etmeye yardımcı olduğu sonucuna varılabilir.

**Anahtar kelimeler:** Baz eksizyon tamiri, D-serin, DNA hasarı, Mefenamik asit

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## INTRODUCTION

Oxidative stress is a phenomenon that occurs with the disruption of the balance between the antioxidant systems in charge of controlling and removing reactive oxygen species (ROS) and the biochemical processes causing the generation of ROS. Excitatory amino acids present at various part of the brain such as glutamate and aspartate, bind to the related receptors and cause them to be overstimulated. By activating various pathways, this situation in turn causes excitotoxicity and may escalate to cell death. D-serine, which acts as the coagonist of N-methyl-D-aspartate (NMDA) receptors and takes part in many physiological and pathological events, is also an amino acid related with excitotoxicity (1-3). Excitotoxicity is associated with cell death that takes place in the acute phase particularly after a stroke or head trauma. In studies conducted in the recent years, the role of excitotoxicity is emphasized also in slow progressing neurodegenerative diseases. In excitotoxicity, with the stimulation of the glutamate receptors in the brain, $Ca^{2+}$ ions pass through the membrane and move into the cell. Increasing intracellular $Ca^{2+}$ levels enhances ROS generation through the mitochondria. The increase in intracellular $Ca^{2+}$ levels triggers the activation of proteases and lipases together with nucleases, which are responsible for the degradation of DNA, and cause cell death (4, 5).

DNA damage means the alterations in the molecular integrity of the genetic material under the effect of endogenous and exogenous factors. DNA damage is a phenomenon experienced frequently by all cells throughout their lives. The damage does not only take place in the nuclear DNA, but also in the mitochondrial DNA (mtDNA). Reviewing the conducted studies shows that mtDNA is more prone to oxidative DNA damage. The facts that the mitochondria are the most significant intracellular source of ROS, that -contrary to nuclear DNA- mtDNA is localized close to the regions generating free radicals in the mitochondria, that mtDNA is not protected via histones and the DNA damage recovery system is inadequate may be listed among the reasons of this (6).

Through various metabolic pathways, the cell develops a response to DNA damage. DNA is repaired in all processes that reduce the genomic instability, cell death, mutation, replication errors and the progression of DNA damage (7). This maintains the cell viability and genomic stability and prevents tumourigenesis. There are approximately 130 genes taking place in DNA repair mechanism.

Nonsteroidal anti-inflammatory drugs (NSAID) have anti-inflammatory, analgesic and antipyretic effects. After it is determined that the native immunity is activated and the inflammation has started to cause damage on organs with low regeneration capacity such as the brain, the studies focused on the use of NSAIDs with neuroprotective purposes for diseases such as Alzheimer, Parkinson, Huntington, multiple sclerosis, stroke and trauma has increased (8). Due to this reason, these drugs show promise in terms of directly or indirectly assisting in the treatment of neurodegenerative diseases.

The primary objective of our study is to evaluate the neuroprotective activity of mefenamic acid as a NSAID on an excitotoxicity experiment model through the enzymes related with DNA repair. For this purpose, the effects of mefenamic acid against D-serine were determined on the expression of base excision repair (BER) enzymes such as 8-oxoguanine DNA glycosylase (OGG1) and apurinic/apyrimidinic endonuclease 1 (APE1), and poly (ADP-ribose) polymerase-1 (PARP-1). The changes in molecules that take place in DNA repair such as PARP-1, APE1 and OGG1 were examined both at mRNA and protein levels in hippocampus of rats. In addition, to evaluate the role of D-serine and mefenamic acid on DNA base damage, 8OHdG levels were determined.

## EXPERIMENTAL

### *Materials and Methods*

*Animals and treatments*

Adult Sprague-Dawley rats weighing 200-240 g were used for the present study. All animals were maintained on a 12:12 h darklight cycle, with free access to chow and water. The protocol for the experiment was approved by the Appropriate Animal Care Committee of Ege University (No. 2009-34). All efforts were made to minimize the number of animals used and their suffering. D-serine was obtained from Sigma-Aldrich (Wien, Austria), original chemical form of mefenamic acid was obtained from Eczacıbası Pharmaceuticals Company (Istanbul, Turkey), and dissolved in physiological saline. The agents were intraperitoneally (i.p.) injected. Animals were divided into four groups: (i) the control group (*n*=6) was given saline only; (ii) D-serine group (*n*=6) was given 200 mg/kg D-serine; (iii) Mefenamic acid group (*n*=6) was given 25 mg/kg mefenamic acid; (iv) D-serine + Mefenamic acid group (*n*=6) was given D-serine (200 mg/kg) and mefenamic acid (25 mg/kg). Mefenamic acid was given 30 min prior to D-serine administration. Rats were decapitated 6 hours after the injections. All animals injected with D-serine were kept in observation for 6 hours to score behavioral alterations. The time (6 hours) and dose (200 mg/kg) of D-serine were chosen according to our previous study in which these dose and time of exposure produced significant oxidative stress in rat brain (9). The dose of mefenamic acid was based upon single therapeutic dose used in humans (10). The hippocampus was dissected on ice. All samples were stored at –80°C until they were used.

*8-hydroxydeoxyguanosine (8-OHdG) measurement*

DNA was isolated from hippocampus using a GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) and DNase-free RNase was used to degrade RNA according to the manufacturer's protocols. Measurement of 8-OHdG was performed with OxiSelect Oxidative DNA damage ELISA kit (Cell Biolabs, Inc.,San Diego, CA). Briefly, DNA was converted to single stranded DNA and 8-OHdG was quantified by quantitative ELISA assay. The quantity of 8-OHdG in hippocampus was determined by comparing its absorbance with known 8-OHdG standard curve.

*Total RNA isolation*

Total RNA was extracted from the hippocampus using TRITidyG reagent (AppliChem, Darmstadt, Germany) followed by phenol-chloroform extraction and isopropanol precipitation. The concentration of RNA in each sample was measured at 260 nm, and its integrity was assessed after electrophoresis (data not shown).

*Reverse transcription PCR*

Prior to the reverse transcription reaction, potentially contaminating residual genomic DNA was eliminated with DNAse I (MBI Fermentas). 1 µg/µL total RNA was used for first strand complementary DNA (cDNA) synthesis by MMuLV reverse transcriptase (MBI Fermentas).

*Real-time PCR*

*PARP-1* (GenBank ID: NM_013063), *APE1* (GenBank ID: NM_024148), *OGG1* (GenBank ID: 030870.1) and *β-actin* (GenBank ID: NM_031144.3) primers were newly designed using Primer3 software. Forward and reverse primers are given in Table 1.

**Table 1.** Specific primer sequences used for real-time PCR analysis

| Target | Forward primer 5'→ 3' | Reverse primer 5'→3' |
|---|---|---|
| ***PARP-1*** **(NM_013063)** | 5' CGC TCA AGG CTC AGA ACG AG 3' | 5' CAG GAT TGC GGA CTC TCC A 3' |
| ***APE1*****(NM_024148)** | 5' TGG AAT GTG GAT GGG CTT CGA CC 3' | 5' ATG GAG CTG ACC AGT ACT GAT GG 3' |
| ***OGG1*** **(NM_030870.1)** | 5' CGC TAT GTA TGT GCC AGT GCT AAA 3' | 5' CCT TAG TCT GCG ATG TCT TAG GCT3' |
| ***B-actin*** **(NM_031144.3)** | 5' AGG GAA ATC GTG CGT GAC AT3' | 5' TCC AGG GAG GAA GAG GAT GC 3' |

Conditions for PCRs were optimized in a gradient cycler (Techne 512, UK) with regard to primers and various annealing temperatures. Optimized settings were transferred to real-time PCR protocols on a Stratagene Mx3000P real-time detection system (Stratagene, La Jolla, CA). Amplification of 1 µL reverse transcription mixture (cDNA diluted 1:5) was carried out using 1 µL (15 pmol) forward and reverse primers, 10 µL Brilliant III SYBR Green Q PCR 2X Master Mix (Stratagene, La Jolla, CA) and 7 µL nuclease-free water in a total volume of 20 µL. Cycling parameters were as follows: 10 min at 95°C, 20 sec at 95°C followed by 40 cycles of 20 sec at 60°C. An additional cycle for melting curve analyses was 1 min at 95°C, 30 sec at 55°C and 30 sec at 95°C. Both cDNA synthesis and PCR amplifications included negative control reactions, which were set up by excluding RNA and DNA templates, respectively. The amplification specificity of the PCR products of *PARP-1, OGG1* and *APE1* were confirmed by the melting curve analysis (data not shown). *B-actin* gene was used as an endogenous control for normalization. The relative expressions of target genes were quantified according to ABI Prism 7700 Sequence Detection System User Bulletin No. 2 (Applied Biosystems, Foster City, CA, USA) and Livak and Schmittengen (11). The relative expression level (fold changes) of mRNAs in hippocampus of mefenamic acid and/or D-serine or saline treated rats were calculated by $2^{-\Delta\Delta CT}$ method. Real-time PCR products were normalized to its corresponding *β-actin* mRNA.

*Isolation of proteins*

The brain region was homogenized in an ice-cold lysis buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulphate, 5 mM EDTA, 1 mM phenylmethylsulphonyl fluoride, 5 mg/mL aprotinin, leupeptin and pepstatin), followed by centrifugation at 12 000×g for 15 min at +4°C. The protein content of the supernatant was measured by using BSA as a standard (12). The isolated protein was subjected to western blotting.

*Western blotting*

Western blotting was performed by loading 10 µg protein on 10% (w/v) tris–glycine denaturing gels and separating proteins by electrophoresis, then transferring to PVDF membrane. After blocking nonspecific binding with the blocking buffer for 2 hours, the membrane was incubated with primary antibodies, anti-PARP-1 mouse monoclonal antibody (1:1000, Santa Cruz Biotechnology), anti-APE1 rabbit polyclonal antibody (1:1000, Cell Signaling Technology) or anti-OGG1 rabbit polyclonal antibody (1:1000, Novus Biologicals) at +4°C over night. Following the incubation with primary antibodies of PARP-1, OGG1 and APE1, the membrane was also incubated with anti-β-actin mouse monoclonal or rabbit polyclonal antibody (1:1000, Santa Cruz Biotechnology) at room temperature for 1 h. After washing, the membrane was incubated with infrared labeled secondary antibodies

(Odyssey Western Blotting kit, LI-COR Biosciences) at room temperature for 1 hour. Proteins were visualized, and quantified by scanning the membrane on an Odyssey Infrared Imaging System (LI-COR Biosciences, USA) with both 700- and 800-nm channels.

*Statistical methods*

To determine the sample size needed in each group, a power analysis was performed based on previously published results using the same model with level of significance of 0.05 and of 0.80 for power (13). The analysis suggested a sample size of 6 in each group. PARP-1, APE1 and OGG1 mRNA or protein contents in the hippocampus of rats treated with D-serine and/or mefenamic acid were calculated as a ratio to the respective control. Thus, the mean values of control group were taken as "1" for other groups. All data present mean ± standard deviation (S.D.) from six animals each group. Differences between the groups were compared by One-Way ANOVA test (Tukey post hoc test). Analysis were performed using the SPSS for Windows 13.0 program. A level of $p<0.05$ was considered to be statistically significant.

## RESULTS

Systemic D-serine administration at 6h produced behavioral changes. Rats injected with 200 mg/kg of D-serine exhibited immobility and rigid postures that were replaced by 'staring spells' after approximately 30 min, followed by repetitive head nodding, 'wet dog shakes' and subsequent rearing and falling. The rats following D-serine treatment showed behaviors that included face-washing behavior and mild convulsions. However, we did not observe any severe tonic convulsions following D-serine treatment. No behavioral abnormalities were observed in the control animals.

In the present study, the levels of 8-OHdG were determined as a marker of oxidative stress-induced DNA base damage. As shown in Figure 1, mefenamic acid treatment did not change 8-OHdG levels in DNA ($p>0.05$). However, 8-OHdG levels were found significantly higher in D-serine and D-serine + mefenamic acid groups ($p<0.01$). Besides, cotreatment of mefenamic acid and D-serine significantly decreased the levels of 8-OHdG when compared with D-serine group ($p<0.01$).

We tested whether mefenamic acid treatment leads to alteration on mRNA levels of base excision repair enzymes and *PARP-1* in hippocampus of rats, alone or with D-serine. Expression levels of genes were analyzed using real-time PCR. To investigate whether gene expression levels are associated with actual protein levels, we used western blotting to examine APE1, PARP-1 and OGG1 protein content in the hippocampus of rats after D-serine or mefenamic acid administration. Systemic administration of D-serine caused significant ($p<0.05$) increase on mRNA levels of *APE1 and PARP-1* (Table 2).

However, there was no difference on *OGG1* mRNA and protein expressions in the hippocampus following treatment with the dose of 200 mg/kg of D-serine. Similar to mRNA levels, APE1 and PARP-1 protein expression levels were significantly increased following D-serine treatment in hippocampus (Figure 2).

Mefenamic acid alone, caused significant ($p<0.05$) increase on mRNA levels of *APE1*, but did not alter *PARP-1* and *OGG1* mRNA levels (Table 2). Although there was a decreasing trend in *PARP-1* mRNA levels, protein expressions of interested enzymes did not significantly change with mefenamic acid alone treatment compared to control and D-serine groups (Figure 2). On the other hand, administration of mefenamic acid against D-serine ameliorated the changes induced by D-serine in hippocampus. mRNA levels of base excision repair enzyme, *APE1,* and *PARP-1* were upregulated following mefenamic acid treatment against D-serine administration (Table 2). Similar to D-serine alone or mefenamic acid alone groups, mRNA levels of *OGG1* did not change following treatment with D-serine and mefenamic acid (Table 1). Correspondingly in western blotting analysis, protein levels of APE1 and PARP-1 were upregulated compared to control group after

coadministration of mefenamic acid and D-serine in hippocampus (Figure 2).

Figure 3 shows the relative densitometric values and relative expression levels of PARP-1 (A), APE1 (B) and OGG1 (C) in hippocampus, respectively. It has been proposed that translational regulation, differences in protein in vivo half-lives, and the significant amount of experimental error, including differences with respect to the experimental conditions are major reasons for the lack of a strong correlation between mRNA and protein expression levels.

conditions, D-serine binds to NMDA receptors in postsynaptic membranes and assists agonists' effects to occur within the cell. It is known that D-serine releases in the presence of agents causing inflammation such as amyloid beta peptide and lipopolysaccharide from microglias and, with the chronic use of drugs such as morphine and in case of ischemia, its release increases from astrocytes and reaches levels that causes toxicity in the brain (15). Since it is known that it is present in high concentrations in parts of the brain where NMDA receptors are

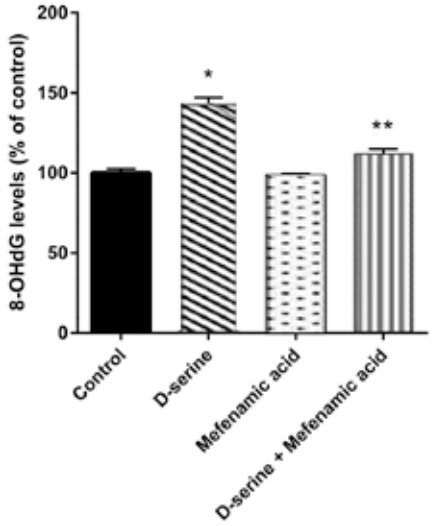


**Figure 1.** Effects of mefenamic acid against D-serine on 8-OHdG levels in the hippocampus. The data are expressed as the mean ± SD, *n*=6. *p<0.001 vs. control; **p<0.01 vs. control or D-serine groups.

## DISCUSSION

Regulation of NMDA receptors has an important role in physiological and pathological processes. Overstimulation of NMDA receptors triggers a series of mechanisms that result in cell death. In addition it has been demonstrated that with the binding of an antagonist on the glycine-binding site, neuronal damage is suppressed (14). Due to this reason it is emphasized in recent years that D-serine as a coagonist may have a role in cell deaths through NMDA receptors. Under physiological

common, the number of studies demonstrating the potential neurotoxic effect of this amino acid is increasing in recent years (16, 17). Although the effect of D-serine on toxicity generation is not exactly known, there are studies indicating that these effects take place through NMDA receptors (17). In addition, it is believed that the metabolites occurring in consequence of D-serine metabolism such as hydrogen peroxide and hydroxypyruvate play a role in D-serine-induced toxicity (18). D-serine's effects on ROS generation, mitochondrial function, lipid peroxidation,

**Table 2.** The relative mRNA expression levels of *PARP-1, APE1* and OGG1 in the hippocampus of rats treated with D-serine and/or mefenamic acid.

| | $2^{-\Delta\Delta C}{}_T \pm$ S.D. | | |
|---|---|---|---|
| | hippocampus | | |
| **Groups** | ***PARP-1*** | ***APE1*** | ***OGG1*** |
| **Control (*n*=6)** | 1.019 ± 0.145 | 1.000 ± 0.028 | 1.054 ± 0.106 |
| **D-serine (*n*=6)** | 1.619 ± 0.212** | 2.345 ± 0.207* | 1.242 ± 0.139 |
| **Mefenamic acid (*n*=6)** | 1.168 ± 0.144 | 1.715 ± 0.127** | 1.338 ± 0.169 |
| **D-serine + Mefenamic acid (*n*=6)** | 2.898 ± 0.159* | 3.068 ± 0.262* | 1.394 ± 0.103 |

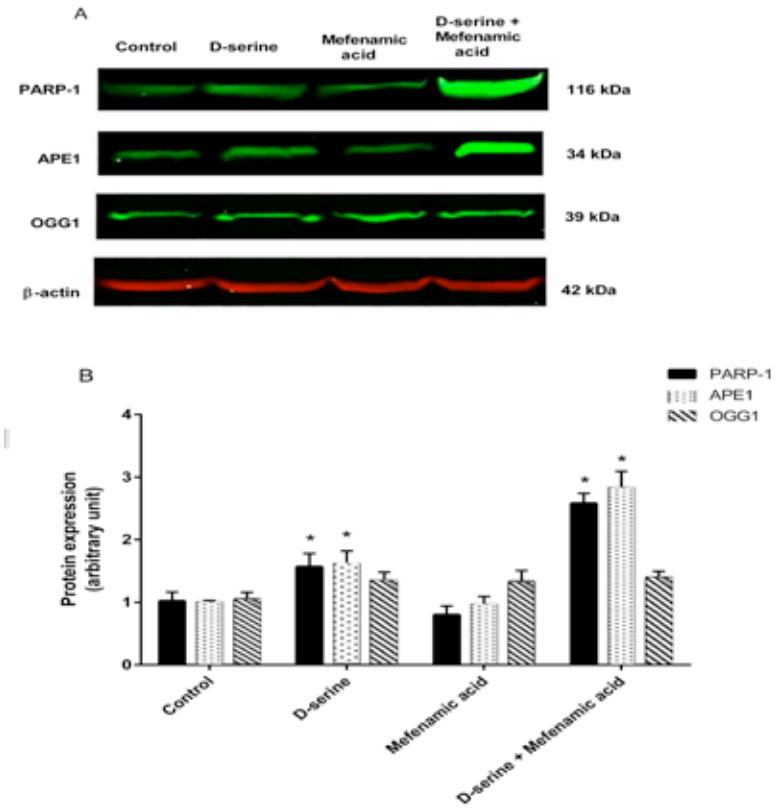


**Figure 2.** Changes in PARP-1, APE1 and OGG1 protein levels in the hippocampus of rats following mefenamic acid treatment against D-serine administration.

Western blot of total protein (50 µg) from hippocampus of rat brain was probed with the primary and secondary antibodies and scanned with a LI-COR® Odyssey® imager. (A) Representative Western blots showing protein expression of PARP-1, APE1 and OGG1. (B) Results indicate the relative densitometric values of PARP-1, APE1 and OGG1 in hippocampus. Quantification of protein product was performed by densitometric scanning. Data are normalized by using the β-actin signal and expressed as arbitrary densitometric units. Values are means ± SD; n = 6 in each group. *p < 0.05 vs control or mefenamic acid groups.

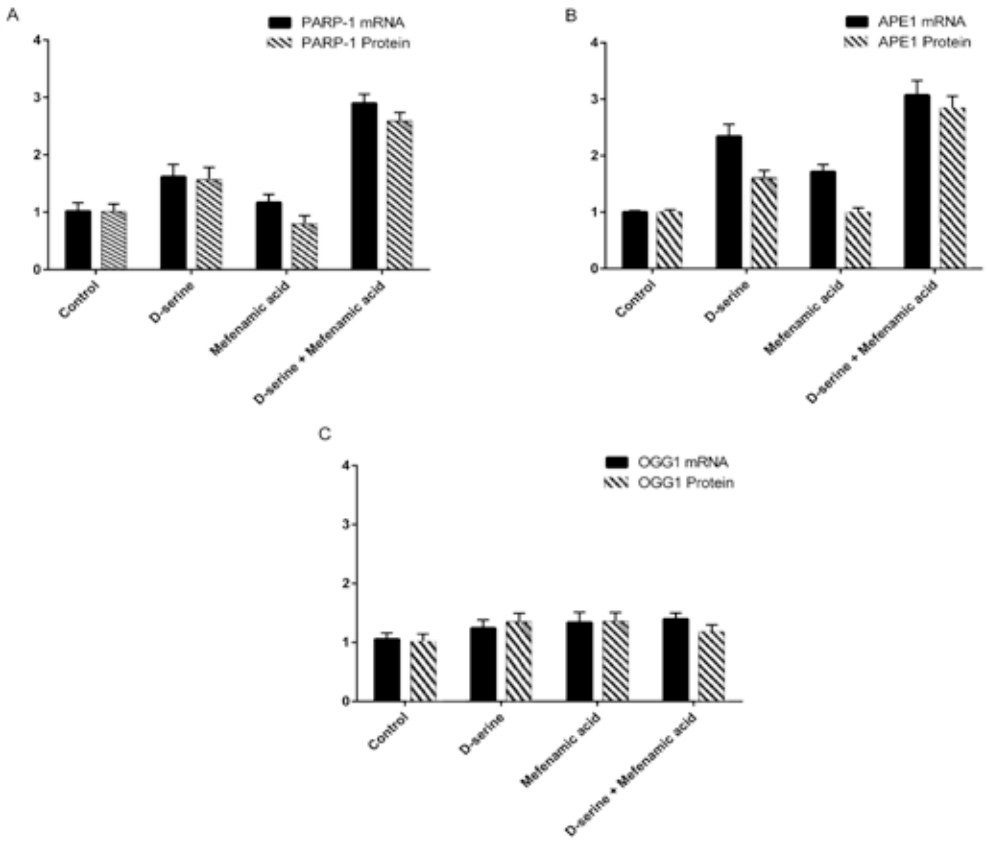


**Figure 3.** The comparison of mRNA and protein levels of PARP-1, APE1 and OGG1 in the hippocampus of rats following mefenamic acid treatment against D-serine administration.

Bar graphs indicate the relative densitometric values and relative expression levels of PARP-1 (A), APE1 (B) and OGG1 (C) in hippocampus, respectively.

protein oxidation and oxidant enzymes were measured in the brains of rats subjected to varying doses and durations of D-serine, and it was demonstrated that D-serine significantly contributes to oxidative stress similar to NMDA and glutamate (9). With regard to the damaging effect on macromolecules such as lipids and proteins, it is considered that high concentrations of D-serine would result in pathological consequences in the brain rather than physiological consequences, and that similarly it may have effects also on DNA damage and/or repair.

Among numerous types of oxidative DNA damage, 8-OHdG formation is an ubiquitous marker of oxidative stress. In the present study, mefenamic acid treatment significantly decreased the levels of 8-OHdG induced by D-serine. These results suggest that mefenamic acid may protect DNA from D-serine-induced oxidative damage.

There are only a limited number of studies that bring together neuroscience and DNA repair mechanisms, despite the fact that the topic is among the popular study areas of the last decade (19). BER proteins with functional disorder may contribute to the pathology of Alzheimer's disease, Parkinson's disease and other neurodegenerative disorders such as stroke (20). Although it is a known fact that

many base excision repair enzymes such as DNA polymerase β, DNA glucosidases and APE1 are synthesized in brain cells, in case of excitotoxicity, increased and unrepaired nuclear DNA damage is in question.

APE1 is a multifunctional protein which plays a part in the base excision repair mechanism and synthesized as a response to DNA damage. It is accepted to act as a neuroprotective protein in the regulation of gene expression and activated in a response to oxidative stress. The activation of glutamate receptors in cerebral cortical neurons causes oxidative DNA damage and it is emphasized that this process also increases the expression of APE1, which is up-regulated through the cAMP-response element binding protein (CREB), and DNA repair takes place. In our study, it was determined that similar to glutamate, D-serine -a NMDA receptor coagonist- increases *APE1* mRNA and protein levels in rat hippocampus tissue. In the treatment of oxidative stress-related diseases, it is aimed to employ agents that cause increase in the levels of proteins in charge of redox regulation of transcription factors in DNA repair and with antioxidant properties such as APE1. It is observed that mefenamic acid against D-serine increases APE1 levels. This is believed to have resulted from the administered mefenamic dosage and that APE1 protein may have a contribution in mefenamic acid's protective effect against D-serine.

The contribution of NMDA receptor activation to the neuroinflammatory processes is well-known. Especially in cases where NMDA receptors are overstimulated such as synaptic excitation and ischemia, it is observed that COX-2 protein and mRNA levels increase rapidly. NSAIDs used under toxic doses exhibit protective effects by inhibiting prostaglandin synthesis. It was demonstrated that the high concentrations of some NSAIDs such as NS-398, ibuprofen and celecoxib reduce the expression of the genes having role in DNA replication and repair (21, 22). Therefore, the potential protective roles of NSAIDs such as mefenamic acid, are still investigated. There are a series of mechanisms such as the cyclooxygenase inhibition, free radical-scavenging, strengthening of the activation of neuronal GABAa receptors and inhibition of $Ca^{2+}$-dependent nonselective cation channels which are believed to have roles in the neuroprotective activity of mefenamic acid. In a study where we examined the effectiveness of mefenamic acid against D-serine, differences in inflammation and apoptosis indicators were tried to be exhibited in various regions of rat brains (13). In that study, it was observed that TNF-α, IL-1β, Bax protein and gene expressions decreased in hippocampus, cortex and cerebellum with the administration of mefenamic acid. Our findings have shown that mefenamic acid may exert its protection through antiapoptotic mechanisms against NMDA receptor activation (13) and these findings support the idea that the fenamates protect cells against the excitotoxic insult (23).

OGG1, one of the DNA glycosylases involved in base excision repair, ensures the recognition and removal of the damaged 8-oxoguanine and FAPY G base in the DNA. In our study it was observed that D-serine did not cause a significant difference in OGG1 mRNA and protein levels in the rat hippocampus. This is due to the fact that even if a difference occurred in the synthesis of this protein, which is known to be synthesized at a low amount in the hippocampus, it may not have been detected with the present method. Another approach that may be the cause of not finding any significant difference in OGG1 levels is the possibility that another DNA glycosylase that is expressed at a higher level in this section such as NEIL1 may play the primary role in removing oxidized bases. Examining mRNA levels in mice brain regions shows that OGG1 is synthesized the most in the cerebellum and in the thalamus. However, mRNA levels of OGG1 in the hippocampus are quite lower than another DNA glycosylase, NEIL1.

Excitotoxicity, inflammation, ischemia and toxins increase DNA damage and accordingly PARP-1 activation. In consequence of PARP-1 activation, DNA repair or cell death takes place depending on the severity of DNA damage. The increased PARP-1 activity in the brain tissue of Parkinson's, Alzheimer's and ALS disease

patients is accepted as the indicator of both increased DNA damage and activated DNA repair mechanisms (24, 25). In studies conducted on excitotoxicity, it was demonstrated that PARP-1 plays an active role in neurotoxicity (26, 27). In our study, we determined that D-serine increases PARP-1 levels in rat hippocampus. While this may be interpreted as PARP-1 contributing to D-serine-induced cell death, it also brings to mind that it may be helping DNA repair enzymes against DNA damage caused by D-serine. While it was expected that PARP-1 levels, which increased in consequence of D-serine application in hippocampus tissue, would decrease with mefenamic acid application due to its anti-inflammatory property, it was actually observed to increase. The fact that PARP-1 mRNA and protein levels that increased in consequence of D-serine application also increased with the application of mefenamic acid indicates that D-serine does not cause very severe DNA damage and that the mechanisms in charge of protecting the cell were activated. Furthermore, it is believed that the dose and exposure time of mefenamic acid was effective on the observed changes. It is stipulated that mefenamic acid doses above this would reverse the observed effects and trigger DNA damage, as it is the case in NSAIDs. Mefenamic acid was shown to attenuate the neurotoxicities induced by amyloid β peptide $(A\beta)_{1–42}$ treatment and the expression of a Swedish double mutation (KM595/596NL) of amyloid precursor protein (Swe-APP) or the C-terminal fragments of APP (APP-CTs) in neuronal cells. It was also shown that mefenamic acid decreases the production of the free radical nitric oxide and reduces cytochrome c release from mitochondria and caspase-3 activation induced by wt-APP, Swe-APP, or APP-CTs in neuronal cells. In addition, mefenamic acid up-regulated expression of the antiapoptotic protein $Bcl\text{-}X_L$ (28). In our previous studies it was observed that ROS production, Bax protein and gene expressions decreased in hippocampus with the administration of mefenamic acid (13). In the study of Zhang et al (29), PARP-1 was shown to activated in PC12 cells following treatment with neurotoxic agents, including the amyloid beta-protein, hydrogen peroxide, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and its active metabolite N-methyl-4-phenylpyridine ($MPP^+$). While PARP-1 activity was increased 5-fold in 1 h and 20-fold in 3 h by $MPP^+$, direct measurement of DNA damage showed no significant increase by 3 h and less than 4-fold by 24 h. These findings indicated that PARP-1 activity is a sensitive and early index of DNA damage following neurotoxic insults (29). PARP-1 is cleaved by caspase-7 and caspase-3 during apoptosis. This cleavage separates the DNA binding domain from the catalytic domain, culminating in the inactivation of the enzyme (30). The increase in PARP-1 expression with the administration of mefenamic acid and D-serine can be a result of inhibition of caspase-3. Mefenamic acid may prevent the cleavage of PARP-1 by inhibition of caspase-3 and cause elevation of expression levels. Our results suggest the idea that while D-serine causes toxicity, it also induces DNA repair mechanisms. Therefore, it is believed that inadequacy or disruption of DNA repair mechanisms render neurons more vulnerable to glutamate-mediated cell deaths.

## CONCLUSION

There is a relation between the functional disorder or genetical defect of proteins in charge of DNA repair, and neurodegenerative diseases. In diseases with excessive glutamate receptor activation such as Alzheimer's disease, this causes failures in the activation of DNA repair mechanisms, defective DNA repairs and accumulation of damaged DNA in brain cells. Due to this reason it is believed that the clarification of various pathways that would trigger DNA repair for the protection of neurons may enable the development of new approaches against damages and neurodegenerative disorders.

## ACKNOWLEDGEMENT

The authors declare that they have no conflict of interest. This study was funded by the Ege

University Scientific Research Foundation (Project No: 11/ECZ/025). The authors also acknowledge the Pharmaceutical Sciences Research Centre (FABAL) of Ege University, Faculty of Pharmacy for equipmental support.

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Received: 10.09.2015
Accepted: 22.10.2015