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Neuroprotective Effects of Dexmedetomidine in Acute Carbon Monoxide Intoxication: An Experimental Study

Akut Karbonmonoksit İntoksikasyonunda Deksmedetomidin'in Nöroprotektif Etkileri: Deneysel Bir Çalışma

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ABSTRACT

Objective: It is aimed to evaluate the neuroprotective effects of Dexmedetomidine (DEX), which has antioxidant, antiapoptotic, anti-inflammatory properties, in acute carbon monoxide toxicity.

Material and Method: 28 Wistar-Albino female rats were randomly divided into four groups as control, Carbon monoxide (CO) poisoning, CO poisoning + DEX and DEX only. The rats in the study groups were exposed to 3000 ppm CO for 30 minutes. DEX was administered half an hour after the onset of CO exposure. At the end of the experiment, blood and tissue samples were taken from the sacrificed rats. Bcl-2 Immunopositively cell values in tissue samples taken from prefrontal and hippocampal areas were scored by examining immune expressions of Bcl-2 antibodies obtained by immunohistochemical method under light microscope. Malondialdehyde (MDA), nitric oxide (NO), asymmetric dimethylarginine ADMA levels, superoxide dismutase (SOD), and catalase (CAT) activity values were measured from blood and right hemisphere brain tissue samples by biochemical methods.

Results: CAT, SOD, MDA, ADMA and NO values were statistically different between the experimental groups (*p<0.001*). According to the post-hoc pairwise comparison test results, there was no statistical difference in any parameter between the DEX group alone and the control group (*p>0.05*). CAT, SOD and NO, and Bcl-2 immunosuppressive cell levels were decreased in the CO group compared to the control group (*p<0.001* in all), while ADMA and MDA levels increased (*p<0.001* in all). CAT, SOD, and NO levels were statistically higher in the CO + DEX group compared to the CO group (*p:0.007; p:0.028; p:0.017, respectively*).

Conclusion: DEX administered half an hour after CO poisoning increases antioxidant structures such as CAT, SOD and NO. Accordingly, DEX may have a neuroprotective effect for carbon monoxide poisoning.

Keywords: Carbon monoxide, dexmedetomidine, neuroprotective effect.

ÖZET

Amaç: Antioksidan, antiapopitotik, antiinflamatuar özellikleri olan Deksmedetomidin'in (DEX) akut karbonmonoksit (CO) toksikasyonunda nöroprotektif etkilerinin değerlendirmesi amaçlanmıştır.

Gereç ve Yöntem: 28 adet Wistar-Albino dişi sıçan kontrol, CO zehirlenme, CO zehirlenme + DEX ve sadece DEX olmak üzere rastgele dört gruba ayrıldı. Çalışma gruplarındaki sıçanlar 3000 ppm konsantrasyonda CO'e 30 dakika boyunca maruz bırakıldı. DEX CO maruziyetinden yarım saat sonra uygulandı. Deney bitiminde sakrifiye edilen sıçanlardan kan ve doku örnekleri alındı. Prefrontal ve hipokampal alanlardan alınan doku örneklerinde Bcl-2 İmmunopositif hücre değerleri immunohistokimyasal yöntem ile elde edilen Bcl-2 antikorların immune ekspresyonlarının ışık mikroskobu altında incelenmesi ile skorlandı. Alınan kan ve sağ hemisfer beyin doku örneklerinden biyokimyasal yöntemlerle malondialdehit (MDA), nitrik oksit (NO), asimetrik dimetilarjinin (ADMA) düzeyleri ile süperoksit düsmutaz (SOD) ve katalaz (CAT) aktivite değerleri ölçüldü.

Bulgular: Deney grupları arasında CAT, SOD, MDA, ADMA ve NO değerleri istatistiksel olarak farklı idi (*p<0,001*). Post-hoc ikişerli karşılaştırma test sonuçlarına göre yalnız DEX grubu ve kontrol grubu arasında hiçbir parametrede istatistiksel fark yoktu (*p>0,05)*. CO grubunda CAT, SOD ve NO ve Bcl-2 immünsüpresif hücre düzeyleri kontrol grubuna göre azaldı (tamamında *p<0,001*) ve ADMA ve MDA düzeyleri arttı (tamamında *p<0,001*). CO + DEX grubunda CO grubuna göre CAT, SOD ve NO düzeylerini istatistiksel olarak daha yüksekti (*sırasıyla p:0,007; p:0,028; p:0,017*).

Sonuç: CO zehirlenmesinden yarım saat sonra uygulanan DEX CAT, SOD ve NO gibi antioksidan yapıları arttırır. Buna bağlı olarak DEX'in CO zehirlenmesi için nöroprotektif bir etkisi olabilir.

Anahtar Sözcükler: Deksmedetomidin, karbonmonoksit, nöroprotektif etki.

Introduction

Carbon monoxide (CO) is a colorless, odorless, and poisonous gas that occurs as a result of incomplete combustion of carbon-based fuels and substances. It is the most common lethal poison worldwide and frequently leads to neurological sequelae as the most common morbidity (1,2). CO has an affinity for hemoglobin (Hb) approximately 250 times that of oxygen. By reversible binding with the iron in hemoglobin, CO forms carboxyhemoglobin (COHb), reducing the capacity of oxygen transport in the blood and decreasing oxygen delivery to tissues (3).

The pathophysiological mechanisms of CO toxicity are classified into hypoxic and cellular theories. CO induces hypoxia by forming COHb and shifting the oxygen-hemoglobin dissociation curve to the left. This leads to hypoxia due to reduced oxygen delivery to tissues, resulting in decreased tissue perfusion and adverse effects on various systems such as cardiac function and neuropsychological function (4). CO poisoning can cause ischemic and hypoxic brain damage in survivors by inducing damage to oxygen distribution and mitochondrial oxidative phosphorylation (5). Brain damage may arise from various factors including neurotoxicity, acidity, electrolyte imbalance, depolarization, oxidative stress, nitric oxide stress, inflammation, and cellular death (6).

Dexmedetomidine (DEX) is a selective α2 receptor agonist routinely used for sedation and anesthetic applications in clinical practice. Unlike most sedatives, DEX induces conscious sedation in patients with its reversible sedative effect, allowing easy arousal (7). Due to its ability to provide conscious sedation, DEX is commonly used in awake craniotomy. Additionally, it possesses anxiolytic and analgesic potentials (8). DEX causes less respiratory depression compared to other sedatives, but there may be an increased risk of hypotension and bradycardia. Particularly, caution should be exercised in using this drug in patients with hepatic impairment due to its involvement in DEX pharmacokinetics (9). However, DEX exhibits neuroprotective effects by reducing inflammation, apoptosis, autophagy, preserving the blood-brain barrier, reducing brain edema, and maintaining cellular structure (10).

The hypothesis of this study is that the administration of DEX to rats exposed to CO poisoning may exert a neuroprotective effect. Based on this hypothesis, this study aimed to evaluate the effects of CO exposure and DEX administration on the activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), the levels of malondialdehyde (MDA) as the end product of membrane lipid peroxidation, asymmetric dimethylarginine (ADMA) levels indicating endothelial damage, and nitric oxide (NO) levels, as well as the distribution of B-cell lymphoma gene 2 (Bcl-2), known as anti-apoptotic and antioxidant, in brain tissue through immunohistochemical analysis. The study aimed to compare these parameters and evaluate the neuroprotective effects of DEX in acute carbon monoxide intoxication.

Material and Method

This study was conducted after obtaining approval from the Local Ethics Committee for Animal Experiments of University of Health Sciences Ankara Training and Research Hospital (Approval No: 2022-0073). This study was conducted in accordance with our country's animal rights regulations and international animal welfare standards. All animals used in the study were cared for in accordance with the highest level of welfare standards before and during the experiment, and all necessary precautions were taken to prevent unnecessary pain, suffering or harm. A total of 28 female Wistar albino rats, weighing an average of 250 grams, were obtained from the Experimental Animals Laboratory of Ankara Education and Research Hospital for use in this study. The carbon monoxide gas utilized in the experiment was prepared by HABAŞ Industrial and Medical Gases Industry Inc. in Izmit, and it contained CO at a concentration of 3000 ppm.

Formation of Experimental Groups

The experimental animals were kept under laboratory conditions and fasted for 12 hours. The rats were randomly selected using a lottery system and grouped to apply the procedures outlined below:

Group 1: Control group (n=7); consisting of 7 rats, placed in the experimental chamber and exposed to room air for 30 minutes. Euthanasia was performed at the end of the 30th minute, and blood and tissue samples were collected.

Group 2: Dexmedetomidine group (n=7); comprising 7 rats, this group received intraperitoneal DEX (10 µg/kg/min) 30 minutes before being placed in the experimental chamber. They were exposed to room air for 30 minutes. Euthanasia was performed at the end of the 30th minute, and blood and tissue samples were collected.

Group 3: CO poisoning group (n=7); consisting of 7 rats, this group was placed in the experimental chamber. Rats placed in the experimental chambers were exposed to CO gas at a concentration of 3000 ppm for 30 minutes at a flow rate of 3 liters/minute, creating CO poisoning. Euthanasia was performed at the end of the 30th minute, and blood and tissue samples were collected.

Group 4: CO poisoning + Dexmedetomidine group (n=7); comprising 7 rats, this group was placed in the experimental chamber and exposed to CO gas at a concentration of 3000 ppm for 30 minutes at a flow rate of 3 liters/minute, creating CO poisoning. Afterward, intraperitoneal DEX (10 µg/kg/min) was administered for 30 minutes. Subsequently, euthanasia was performed, and blood and tissue samples were collected.

Immunohistochemical Analysis

In this study, Tissues obtained from the prefrontal and hippocampal regions were fixed in formalin and left for at least one day before undergoing paraffin embedding. Paraffin embedding involved tissue tracking, followed by sectioning with a microtome to obtain 5 µm thick sections, which were then placed on slides. These sections were then incubated overnight at 54°C. After incubation, the sections underwent two rounds of 20-minute deparaffinization in xylene to remove paraffin. Subsequently, the sections were rehydrated by passing through a series of decreasing alcohol concentrations (100%, 90%, 80%, and 70%). Following rehydration, the sections were washed twice with fetal calf serum (PBS) and then subjected to microwave irradiation in 250 ml freshly prepared citric acid buffer (pH: 6.0) to expose antigenic epitopes. After microwave treatment, the sections were cooled at room temperature for 20 minutes and then washed three times for five minutes each with PBS at room temperature.

Next, to saturate endogenous peroxidase activity in the tissue, the sections were incubated in a 3% H_2O_2 solution at room temperature for 20 minutes. The sections were washed three times for five minutes each with PBS and then incubated with blocking solution at room temperature for 7 minutes to block nonspecific bindings. After removal of the blocking solution, the samples were incubated overnight at +4°C in a humid environment with anti-bcl-2 antibodies. Appropriate isotype control antibodies or sera were used as negative controls, ensuring they were of the same concentration as the primary antibodies. Following incubation with the primary antibodies, the sections were subjected to three 5-minute washes and the sections were then incubated with biotinylated secondary antibodies for 45 minutes at room temperature.

After the incubation with biotinylated secondary antibodies, the tissue samples were incubated with streptavidin-peroxidase complex at room temperature for 30 minutes. After washing with PBS, diaminobenzidine solution was added to visualize the antigen-antibody complexes, and after washing with water, they were counterstained with Mayer's hematoxylin. The samples were then dehydrated through increasing alcohol series, clarified in xylene, and cover slipped with mounting solution. Subsequently, the chromogenic reaction of the antibodies was examined for immunoexpressing under a light microscope. Immunoexpressing was scored semi-quantitatively as 0 for no expression, 1 for weak expression, 2 for moderate expression, and 3 for strong expression. Statistical analysis was performed to evaluate whether there was a difference between groups using the Mann-Whitney U test.

Collection and Preparation of Blood and Tissue Samples

The rats were sacrificed, and blood samples were collected via intracardiac puncture into biochemical tubes. The biochemical blood samples were centrifuged at 1500 rpm for 10 minutes to separate the sera, which were aliquoted.

Brain tissues were removed, washed with saline solution, and the right hemispheres were weighed. Then, they were immediately frozen in liquid nitrogen and stored at -80°C.

At the time of analysis, all tissue samples were homogenized in 1/6 phosphate buffer (pH: 7.4) using a blade homogenizer, and then centrifuged at 20,000

Table I. Comparison of laboratory parameters according to rat groups by ANOVA test

	Control (1)	Dex (2)	CO (3)	Dex + CO (4)	*P*	*Post-hoc P*
CAT (IU/ml)	4.21±0.89	4.47±0.3	1.22±0.50	3.02±0.94	*<0.001**	1-3: <0.001 2-3: <0.001 *2-4: 0.033* *3-4: 0.007*
SOD (U/ml)	1.13±0.17	1.34±0.35	0.39±0.14	0.85±0.30	*<0.001**	*1-3: <0.001* *2-3: <0.001* *2-4: 0.018* *3-4: 0.028*
MDA (nmol/ml)	1.13±0.25	1.32±0.29	2.48±0.51	2±0.40	*<0.001**	*1-3: <0.001* *1-4: 0.004* *2-3: <0.001* *2-4: 0.030*
ADMA (µmol)	119.7±70.42	129.3±41.85	359.8±87.75	299.2±88.09	*<0.001**	*1-3:<0.001* *1-4: 0.002* *2-3<0.001* *2-4: 0.004*
NO (µmol/g)	52.87±8.99	47.05±9.08	24.10±8.36	39.1±3.83	*<0.001**	1-3: <0.001 1-4: 0.031 2-3: <0.001 3-4: 0.017

*: ANOVA ve Post-hoc Tukey test

rpm for 10 minutes to separate the supernatants.

Biochemical Parameters

Total protein levels in all homogenates and supernatants were determined spectrophotometrically using the Bradford method. MDA levels in homogenates were assessed using the ELISA method. The activity levels of SOD and CAT in supernatants were determined using commercial kits, whereas NO and ADMA levels were determined using the ELISA method. To normalize enzyme activity values, they were divided by the protein values in the supernatants.

Statistical Analysis

Statistical analyses were performed using the SPSS program (Version 22.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard deviation. The normal distribution of groups was assessed using the Shapiro-Wilk test. Since the data were normally distributed, statistical comparison of numerical measurements among independent groups of more than two was conducted using one-way analysis of variance (ANOVA). Post-hoc Tukey test was performed for pairwise comparisons to determine which groups showed differences after ANOVA. A *p-value < 0.05* was considered statistically significant.

Results

According to the results of the ANOVA test, CAT, SOD, MDA, ADMA, and NO values were statistically significantly different among the experimental groups (*p<0.001*). Post-hoc pairwise comparison test results revealed that CAT and SOD values in the control and DEX groups were significantly higher than those in the CO group. Additionally, CAT and SOD values in the DEX group were significantly higher than those in the DEX + CO group. CAT and SOD values in the CO group were significantly lower than those in the DEX + CO group (Table I).

Table II. Comparison of Bcl-2 Immunopositive cells values according to rat groups by ANOVA test

Control (1)	Dex (2)	CO (3)	Dex+ CO (4)	*P*	Post-hoc P	
Bcl-2 İmmunopositive cells (U/ml)	31.07±6.83	25.35±5.81	13.42±3.76	18.18±3.85	*<0.001**	1-3: <0.001 1-4: 0.002 2-3: 0.004

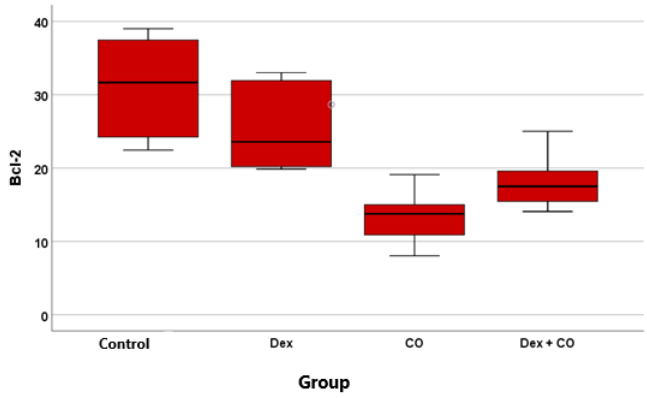
*: ANOVA ve Post-hoc Tukey test

According to post-hoc pairwise comparison test results, MDA and ADMA values in the control and DEX groups were significantly lower than those in the CO group. Additionally, MDA and ADMA values in the DEX group were significantly lower than those in the DEX + CO group. MDA and ADMA values in the CO group were significantly higher than those in the DEX + CO group (Table I).

Based on the post-hoc pairwise comparison test results, it was found that NO values in both the control and DEX groups were significantly higher compared to the CO group. Furthermore, NO values in the control group were notably higher than those in the DEX + CO group. Conversely, NO values in the

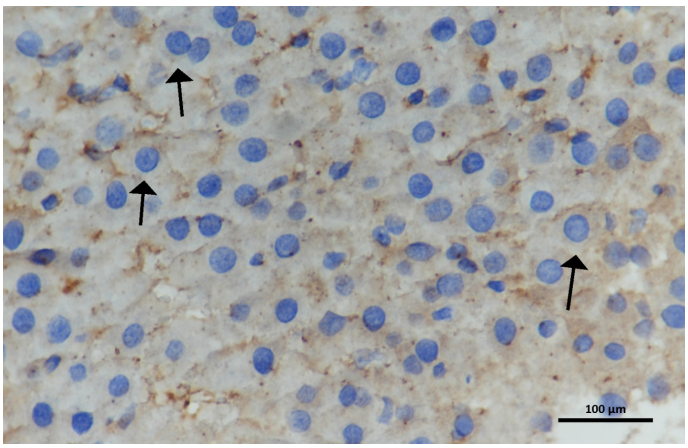
CO group were significantly lower than those in the DEX + CO group (Table I).

Figure I. Box plot showing the distribution of Bcl-2(U/ml) immunopositive cells between groups



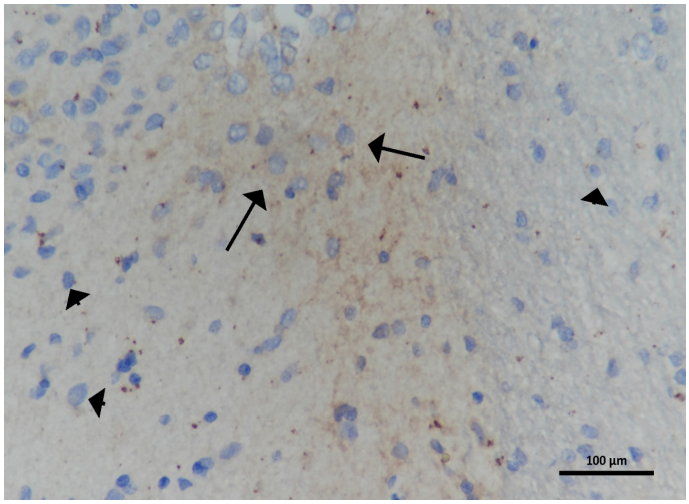
According to the results of the ANOVA test, there were statistically significant differences in the values of Bcl-2 immunopositively cells among the experimental groups (*p<0.001*). Post-hoc pairwise comparison test results showed that Bcl-2 immunopositively cells values in the control and DEX groups were significantly higher than those in the CO group. Additionally, Bcl-2 immunopositively cells values in the control group were significantly higher than those in the DEX + CO group (Table II). The distribution of Bcl-2 immunopositively cells values among groups is presented in Figure I.

Figure II. Cells stained strongly positive for anti-bcl-2 in control group brain section (arrow), bar 100 µm



In the immunostaining conducted with the anti-Bcl-2 antibody in the hippocampus, widespread expression of Bcl-2 (indicated by arrows) was observed in the control group. (Figure II). The DEX + CO group brain section showed areas with moderate positive staining of anti-bcl-2 (arrow) and areas with no staining (arrowhead) (Figure III).

Figure III. Area with cells stained moderately positive for anti-bcl-2 (arrow) and unstained cells (arrowhead) in DEX + CO group brain section, bar 100 µm



Discussion

In this study examining the consequences of CO poisoning, the neuroprotective effects of DEX application were assessed. After CO poisoning, CAT, SOD, and NO levels significantly decreased, while MDA and ADMA levels significantly increased. However, in rats treated with DEX after CO poisoning, CAT, SOD, and NO levels were found to be significantly higher compared to rats exposed to CO poisoning alone. Therefore, it is considered that DEX contributes to the antioxidant response following CO intoxication. MDA is the end product of arachidonic acid and larger polyunsaturated fatty acids, decomposed via enzymatic or non-enzymatic pathways. MDA shows a good correlation with the degree of lipid peroxidation (11). According to some studies, MDA functions both as a regulator and a messenger of gene expression. Excessive MDA production has been considered an indicator of oxidative stress in some research, and it has been associated with various pathological conditions such as Alzheimer's disease, cancer, cardiovascular diseases, diabetes, liver diseases, and Parkinson's (12-13). In our study, plasma MDA levels, indicative of oxidative stress, significantly increased after CO exposure, while this increase was not statistically significant with DEX administration, although it decreased.

Antioxidants are specialized defense systems in the body. For a compound to be a good antioxidant, it should not be converted into a reactive radical and should have a specific target. The most important group of antioxidants is SOD. SOD utilizes free radicals as substrates and converts superoxide into H_2O_2. The SOD enzyme is an enzyme that helps protect against the harmful effects of oxygen and superoxide radicals. The distribution of this enzyme should be investigated together with CAT because the product formed after the reaction catalyzed by SOD needs to be removed by CAT to prevent the accumulation of oxygen, which is one of the toxic species. Therefore, it is important to examine the distribution of this enzyme with CAT. CAT is found in peroxisomes and is effective on H_2O_2. CAT accelerates the conversion of H_2O_2 into water and oxygen. SOD and CAT enzymes are considered among the most active antioxidant enzymes in living organisms and are found in all tissues, cells, and intracellular organelles of aerobic organisms. These enzymes play an important role in the maintenance of life (14). Some studies have shown that individuals with low CAT levels are more susceptible to diseases such as type 2 diabetes and hypertension and are affected by diseases such as thickening of arterial walls and tumor formation (15). In our study, it was observed that the antioxidant enzymes CAT and SOD decreased significantly after CO intoxication, and that these enzymes increased significantly after DEX administration following CO intoxication.

NO, which has important functions including vasodilatation and release of inflammatory cytokines and is synthesized by the endothelium, has complex functions in various tissues. Among these functions, it is involved in immunity, nonspecific immunity, inhibition of viral replication and transplant rejection (16). In the nervous system, NO is associated with memory, learning, epilepsy, pain sensitivity and neurodegeneration. In the respiratory system, it is involved in bronchodilatation, asthma and ARDS pathogenesis. In our study, NO levels decreased statistically significantly in rats with CO intoxication but increased statistically significantly in rats given DEX after CO intoxication.

ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), suppressing its activity. As ADMA levels increase, nitric oxide (NO) production decreases. It has been reported that ADMA-induced endothelial dysfunction plays a role in atherosclerosis and can be used as a marker (17). High ADMA levels in patients are believed to result from endothelial insufficiency. The mechanism of endothelial dysfunction caused by ADMA involves reduced vascular NO production and increased vascular superoxide levels (18). In our study, while ADMA levels, indicating endothelial damage, statistically significantly increased in rats with CO intoxication, this increase was statistically not significant after DEX application following CO intoxication.

Bcl-2 is an intracellular membrane protein and is also described as anti-apoptotic and antioxidant. The distribution of this protein differs according to cell type. Bcl-2 is known to have antioxidant properties by reducing lipid peroxidation and to provide resistance against H_2O_2-induced apoptotic death. One of the most widely accepted is that Bcl-2 is a substance that acts as an antioxidant and reduces ROS production (19). Studies conducted to investigate the expression and amount of bcl-2 in different regions of the brain and in various organs and tissues show contradictory results in the natural aging process. This may be due to differences in the cell-specific activity of bcl-2 and survival conditions (20). Kaufmann et al. showed that bcl-2 levels were increased in the hippocampus and cerebellum of aged rats and this increase may be related with the increased age-related oxidative stress level and antioxidant properties of bcl-2 (21). In our study, it was observed that Bcl-2 level, which is also known as anti-apoptotic and antioxidant, decreased significantly in rats given CO, whereas this decrease was more limited with DEX administration after CO intoxication, although not statistically significant.

Although CO intoxication is a common condition, research is ongoing for its treatment. Zengin et al. investigated the neuroprotective effects of amantadine after CO intoxication (22). In their study, they found a statistically significant decrease in NMDA levels with amantadine administration after CO intoxication compared to CO intoxication. Although CAT, SOD, ADMA, NO and Bcl-2 levels were not statistically significant, clinically significant results were obtained. Investigation of oxidant and antioxidant systems,

determination of the severity of poisoning, prediction of post-poisoning outcomes and the search for new markers that can be used as well as the choice of treatment are also important in toxicology. There is evidence that acute antioxidant augmentation may be effective as a new therapeutic strategy to prevent delayed neurological problems in CO poisoning, that CO-mediated brain damage is a result of free radical cascade and that the balance between the antioxidant system and oxidative stress is in a strong relationship (23). Several mechanisms have been associated with the neuroprotective function of dexmedetomidine, including neurotransmitter regulation, inflammatory response, oxidative stress, apoptotic pathway, autophagy, mitochondrial function and other cell signalling pathways. Therefore, dexmedetomidine has been reported to have the potential to be a novel neuroprotective agent for a wide range of neurological disorders (24). Also Chen L et al; DEX protects against diabetic hyperglycaemia-induced cerebral I/R injury through attenuation of oxidative stress, inflammation and apoptosis (25).

In conclusion; when brain mechanisms are considered, brain damage caused by many causes such as hypoxia, free oxygen radicals, inflammation and cell destruction after CO exposure is very complex. The decrease in Bcl-2 level after CO exposure shows that cell destruction increases and the increase in ADMA, which indicates endothelial damage, shows that vascular structures are also impaired after hypoxia. DEX administration has a limited effect to prevent this process in CO poisoning. Dexmedetomidine administered half an hour after CO poisoning increases antioxidant structures such as CAT, SOD and NO. Accordingly, DEX may have a neuroprotective effect for CO poisoning.

More comprehensive studies are required to better understand CO intoxication and its treatment and to evaluate the neuroprotective effects of DEX and the relationship between the oxidant and antioxidant system with CO intoxication and hypoxia. This will lead to new developments in the treatment of CO intoxication.

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