Ameliorating Role of Caffeic Acid Phenethyl Ester (CAPE) Against Methotrexate-Induced Oxidative Stress in the Sciatic Nerve, Spinal Cord and Brain Stem Tissues of Rats

Ratların Siyatik Sinir, Spinal Kord ve Beyin Sapında Metotreksatın Neden Olduğu Oksidatif Strese Karşı Kafeik Asit Fenetil Esterin Koruyucu Rolü

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ÖZET

Amaç: Kanser hastalarında metotreksata bağlı nörotoksisite önemli klinik bir problemdir. Fakat metotreksatın (MTX) neden olduğu nörotoksisitenin mekanizması tam olarak bilinmemektedir. Bu çalışmanın amaçları; MTX'e bağlı nörotoksisitenin patogenezinde malondialdehid (MDA), süperoksid dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve katalaz (CAT)'ın olası rolü ile ratların siyatik sinir, beyin sapı ve medulla spinalisinde MTX'e bağlı nörotoksisitesinde koruyucu etkisi olup olmadığını araştırmaktır.

Gereç ve Yöntem: Toplam 19 adet Wistar erkek rat üç deney grubuna ayrıldı. Grup 1: Kontrol grubu, Grup 2: MTX alan grup, Grup 3: MTX ve kafeik asit fenetil ester (CAPE) alan grup. MTX ve MTX + CAPE gruplarına deneyin ikinci gününde MTX 20 mg/kg tek doz periton içine verildi. MTX CAPE grubuna CAPE 10 µmol/kg/gün intraperitoneal olarak 7 gün verildi.

Bulgular: MTX grubunda siyatik sinir ve spinal kord dokusunda kontrol grubu ile karşılaştırıldığında CAT ve GSH-Px aktivitelerinde artış bulundu. MTX + CAPE ile MTX grubu karşılaştırıldığında CAT ve GSH-Px aktivitelerinde azalma saptandı. MTX grupta spinal kord ve beyin sapı dokularında SOD aktivitesi kontrolle karşılaştırıldığında azalma saptanırken, siyatik sinirde anlamlı fark bulunmadı. Spinal kord ve beyin sapı dokularında SOD aktivitesi MTX + CAPE grubunda MTX grupla karşılaştırıldığında anlamlı derecede artış bulundu. MDA seviyesi MTX grupta kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksekti. MTX + CAPE grubunda MDA seviyeleri MTX grubuna göre istatistiksel olarak anlamlı derecede düşük bulundu.

Yorum: Bu sonuçlar MTX'in rat siyatik sinir, medulla spinalis ve beyin sapında oksidatif stresi artırdığını ve CAPE'nin antioksidan etkisi nedeniyle oksidatif strese karşı koruyucu etkisini gösterir.

Anahtar Kelimeler: Kafeik asit fenetil ester, metotreksat, oksidatif stres, siyatik sinir, medulla spinalis.

ABSTRACT

Ameliorating Role of Caffeic Acid Phenethyl Ester (CAPE) Against Methotrexate-Induced Oxidative Stress in the Sciatic Nerve, Spinal Cord and Brainstem Tissues of Rats

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Objective: Methotrexate (MTX)-associated neurotoxicity is an important clinical problem in cancer patients, but the mechanisms of MTX-induced neurotoxicity are not yet known exactly. The aims of this study were (1) to investigate the possible role of malondialdehyde (MDA), superoxide dismutase (SOD) enzyme, glutathione peroxidase (GSH-Px) and catalase (CAT) in the pathogenesis of MTX-induced neurotoxicity and (2) to determine whether there is a putative protective effect of caffeic acid phenethyl ester (CAPE) on MTX-induced neurotoxicity in the spinal cord, brainstem and sciatic nerve of rats.

Materials and Methods: A total of 19 adult Wistar male rats were divided into three experimental groups. Group I, control group; Group II, MTX-treated group; and Group III, MTX + CAPE-treated group. MTX was administered to the MTX and MTX + CAPE groups intraperitoneally (IP) with a single dose of 20 mg/kg on the second day of the experiment. CAPE was administered to the MTX + CAPE group IP with a dose of 10 µmol/kg for 7 days.

Results: In the sciatic nerve and spinal cord tissue, CAT and GSH-Px activities were increased in the MTX group in comparison with the control group. CAPE treatment with MTX significantly decreased CAT and GSH-Px activities in the neuronal tissues of rats in comparison with the MTX group. In the spinal cord and brainstem tissues, SOD activity in the MTX group was decreased in comparison with the control group, but in the sciatic nerve, there was no significant difference. In the spinal cord and brainstem of rats, SOD activity was increased in the CAPE + MTX group when compared with the MTX group. The level of MDA was higher in the MTX group than in the control group. CAPE administration with MTX injection caused a significant decrease in MDA level when compared with the MTX group.

Conclusion: These results reveal that MTX increases oxidative stress in the sciatic nerve, spinal cord and brainstem of rats and that CAPE has a preventive effect on the oxidative stress via its antioxidant capacity.

Key Words: Caffeic acid phenethyl ester, methotrexate, oxidative stress, sciatic nerve, spinal cord.

INTRODUCTION

Methotrexate (MTX) is a cytotoxic chemotherapeutic agent that is widely used for various malignancies like acute lymphoblastic leukemia, lymphoma, and solid cancers, autoimmune diseases such as rheumatoid arthritis, and multiple sclerosis (1,2). MTX-related neurotoxicity is an important clinical problem in patients (3-5). MTX may affect the neuronal tissues including the brain stem, spinal cord and peripheral nerve, depending on the given dose, route of administration and simultaneous use of other potential neurotoxic agents (3,6-8). Although underlying mechanisms in MTX-induced toxicity are not yet exactly known, diverse hypotheses have been postulated, among which oxidative stress was claimed (7,9). Some possible action pathways have been suggested for MTX neurotoxicity as follows: direct toxic effect of MTX on central and peripheral nervous systems (CNS, PNS), inhibition of several enzymes related to DNA synthesis, increased oxidant homocysteine and decreased antioxidant S-adenosylmethionine levels in blood and cerebrospinal fluid, and MTX-induced oxidative stress in cellular membrane phospholipids of the CNS (4,5,9-11).

The implication of oxidative stress in a wide range of neurological disorders such as seizures, Parkinson's disease and Alzheimer's disease has led to efforts to interfere with the progression of neurodegeneration by antioxidant treatment (12-14). Furthermore, oxidative stress caused by some antineoplastic and chemical toxic agents is known to be an important factor for neuronal toxicity (7,15). Previous studies have indicated that MTX administration induces oxidative stress in the intestinal mucosa, liver, kidney, cerebellum, and spinal cord tissues of rats (7,16-18). Reactive oxygen species (ROS) are produced constantly in cells because of both oxidative biochemical reactions and external factors (19). However, they become detrimental when they are excessively produced in some abnormal conditions including inflammation, drug toxicity, spinal cord injury, and stress (7,19,20). In this case, endogenous antioxidants [e.g. superoxide dismutase (SOD) enzyme, glutathione peroxidase (GSH-Px) and catalase (CAT)] may be unab-

le to block ROS formation, which in turn can lead to cellular damage by lipid peroxidation, sulfhydryl enzyme inactivation, protein crosslinking, and DNA breakdown (21-23). MTX-induced cellular injury occurs through deleterious effects of this agent on DNA, proteins, lipids, and other cellular structures. It has been reported that there was a MTX-related reduction in effectiveness of the antioxidant enzyme defense system (7,9,24). In a previous study, it was reported that there was a decrease in cellular levels of glutathione by MTX (24). Glutathione is known as an important antioxidant and protective substance against ROS (24,25). With respect to the relationship between oxidative stress and the undesired effects of MTX, interest has been focused on antioxidant compounds [e.g. caffeic acid phenethyl ester (CAPE)].

In various researches, CAPE has been extensively used as an antioxidant agent (8,25). CAPE is one of the main components of honeybee propolis and likely has no harmful effects on normal living cells (26). CAPE has an antioxidant effect (25,26). Moreover, anti-inflammatory, anticarcinogenic, neuroprotective, and antiepileptic effects have also been reported (12,27,28). It was suggested that using CAPE in combination with anticancer drugs was protective against anticancer toxicity such as caused by MTX and doxorubicin (7,25,29). The aims of this study were to investigate the possible role of SOD, CAT and GSH-Px activities and malondialdehyde (MDA) levels in the pathogenesis of MTX-induced neurotoxicity and to disclose whether there is a preventive effect of CAPE on the neurotoxic effect of MTX.

MATERIALS and METHODS

Animals and Experiment

A total of 19 male Wistar albino rats (aged 8-12 weeks) weighting between 200-250 g obtained from the Laboratory Animal Production Unit of Suleyman Demirel University were used in the experiment. The rats were divided, maintained and used in conformity with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by Suleyman Demirel University, Animal Ethical Committee." Rats were randomly divided into three experimental groups as follows: 1) MTX group (n= 6): MTX (Ebewe Arzneimittel Ges. M.b.H Pharmaceutical Laboratories, Unterach, Australia) was administered on the second day of our study with a single dose of 20 mg/kg intraperitoneally (IP); 2) MTX + CAPE group (n= 7): CAPE (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) was injected with the dose of 10 µmol/kg IP for five days in addition to MTX with the same dose as in the MTX group; and 3) control group (n= 6): isotonic saline solution (an equal volume of CAPE) was administered IP for five days (7,12,16). Rats were kept in an environment with controlled temperature (24-26°C), humidity (55-60%), and controlled photoperiod (12 h light/dark cycle) for one week before the initiation of the experiment. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum. This experiment lasted for five days.

At the end of the study, all rats were anesthetized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and sacrificed 24 hours after the last administrations, and the brain stem, spinal cord and sciatic nerve tissues were quickly removed. The brain stem, spinal cord and sciatic nerve tissues were stored at -20°C until the analysis of MDA levels and the measurements of SOD, CAT and GSH-Px activities.

Biochemical Procedure

The frozen tissue samples of the brain stem, spinal cord and sciatic nerve tissues of the rats were thawed, weighed, and homogenized (Ultra Turrax T25, Germany) (1/10, w/v) in 50 mM/L phosphate buffer (pH 7.4) kept in an ice bath. The homogenate was then centrifuged at 5000 g for 30 minutes (min) to obtain supernatants. The homogenate and supernatant of tissues were frozen at -20°C in aliquots until they were used for biochemical analysis. The protein content of the homogenate and supernatant was determined using the Lowry method (30). MDA is a marker of free radical generation, which increases at the end of the lipo peroxidation. MDA levels were estimated by the double heating method of Draper and Hadley (31). The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000 g for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7 g/L. TBA solution was placed in a test tube and the tube was placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA-TBA complex (absorbance coefficient ε= 1.56 x 105 cm^{-1} M^{-1}) and was expressed as nanomoles per gram wet tissue. Activity of total (Cu-Zn and Mn) SOD enzyme was measured according to the method of Sun et al. (32). The standard of the method was based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per gram protein.

Activity of CAT enzyme was determined according to the technique of Aebi (33). The principle of the assessment was based on the determination of the rate constant *k* (dimension: s^{-1}, *k*) of hydrogen peroxide decomposition. By measuring the absorbance change per minute, the rate constant of the enzyme was determined.

Activity of GSH-Px was determined by the technique of Paglia and Valentine (34). The enzymatic reaction in the tube that contained reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide, and glutathione reductase was initiated by the addition of hydrogen peroxide, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity of GSH-Px was obtained in units per gram protein. All samples were assessed in duplicate.

Histopathological Procedures

The samples of the sciatic nerve were fixed in 10% neutral buffered formalin and stored at 4°C for one week. Samples were then removed and placed in fresh fixative. Fixed tissue samples were processed routinely by paraffin embedding technique. The sagittal sciatic nerve sections of 5 µm thickness were cut with microtome at 200 µm intervals, and every 10^{th} section through the sciatic nerve was collected on a slide and stained with hematoxylin and eosin (H&E). Three areas were evaluated on each slide and average values were calculated. Preparations were evaluated by a bright field microscope and were photographed (Nikon Microscope ECLIPSE E600W, Tokyo, Japan) and photographed using a digital camera (Microscope Digitale Camera DP70, Tokyo, Japan). The photographs were analyzed by image analysis system. Schwann cells nuclear count in each group was determined in per areas of each section (three areas per section). An area was defined as 255 mm (W) X165 mm (H), in sciatic nerve were calculated by image analysis system. The investigator performing these measurements was blinded to the experimental condition.

A system of a PC, hardware and software was used (the images were processed by an IBM-compatible personal computer, high-resolution video monitor and image analysis software; BS200Docu Version 2.0, BAB Imaging Systems, Ankara, Turkey). The method requires preliminary software procedures of spatial calibration (micron scale) and setting of color segmentation for quantitative color analysis. In the evaluation of the sections, the distribution of vascular proliferation, intensity of congestion and myelin degeneration in the sciatic nerve were scored as 0-4 (no, low, moderate, high and very high, respectively) semi-quantitatively (7).

Statistical Analysis

Data were presented as means ± standard deviation (SD). A computer program (SPSS 9.0) was used for statistical analysis. The one-way analysis of variance (ANOVA) and post hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the difference between each group. A p value of < 0.05 was considered as statistically significant.

The data of histopathological changes were considered to be non-parametric; therefore, they were performed using Kruskal-Wallis H test. Differences between the two groups were determined with Mann-Whitney U test. A value of $p< 0.05$ was considered statistically significant.

RESULTS

Biochemical Results

All rats survived without major complications. Results are shown in Tables 1-3. In the MTX group, MTX administration produced a significant increase in the level of MDA (marker of lipo peroxidative stress) in the brain stem, spinal cord and sciatic nerve tissues when compared with the other groups (p= 0.0001, p= 0.003, p=0.0001, respectively). In the MTX + CAPE group, MTX-induced increments in MDA levels in the brain stem, spi-

Table 1. Sciatic nerve oxidant/antioxidant status in MTX, MTX + CAPE and control groups in rats

Groups	SOD (U/g protein)	CAT (k/g protein)	GSH-Px (U/g protein)	MDA (nmol/w tissue)
(I) Control (n= 6)	0.735 ± 0.147	0.246 ± 0.041	6.201 ± 2.404	271.56 ± 48.07
(II) MTX (n= 6)	0.833 ± 0.157	0.417 ± 0.048	14.259 ± 3.284	411.62 ± 65.65
(III) MTX + CAPE (n= 7)	0.740 ± 0.158	0.249 ± 0.026	9.517 ± 1.257	276.06 ± 37.14
p values				
I-II	NS	0.0001	0.0001	0.0001
I-III	NS	NS	0.016	NS
II-III	NS	0.0001	0.002	0.0001

NS: Statistically insignificant, MTX: Methotrexate, CAPE: Caffeic acid phenethyl ester, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 2. Spinal cord oxidant/antioxidant status in MTX, MTX + CAPE and control groups in rats

Groups	SOD (U/g protein)	CAT (k/g protein)	GSH-Px (U/g protein)	MDA (nmol/g wet tissue)
(I) Control (n= 6)	0.423 ± 0.057	0.078 ± 0.040	5.62 ± 0.75	47.23 ± 6.57
(II) MTX (n= 6)	0.288 ± 0.029	0.211 ± 0.045	8.80 ± 1.15	63.49 ± 8.62
(III) MTX + CAPE (n= 7)	0.401 ± 0.074	0.082 ± 0.022	6.86 ± 0.84	46.95 ± 7.79
p values				
I-II	0.002	0.0001	0.0001	0.003
I-III	NS	NS	0.022	NS
II-III	0.005	0.0001	0.002	0.002

NS: Statistically insignificant, MTX: Methotrexate, CAPE: Caffeic acid phenethyl ester, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 3. Brain stem oxidant/antioxidant status in MTX, MTX + CAPE and control groups in rats

Groups	SOD (U/mg protein)	MDA (nmol/g wet tissue)
(I) Control (n= 6)	0.149 ± 0.007	5.149 ± 0.37
(II) MTX (n= 6)	0.168 ± 0.006	7.301 ± 0.31
(III) MTX + CAPE (n= 7)	0.153 ± 0.009	4.950 ± 0.49
p values		
I-II	0.001	0.0001
I-III	NS	NS
II-III	0.004	0.0001

MTX: Methotrexate, CAPE: Caffeic acid phenethyl ester, SOD: Superoxide dismutase, MDA: Malondialdehyde.

nal cord and sciatic nerve tissues were significantly prevented by CAPE treatment (p= 0.0001, p= 0.002, p= 0.0001, respectively). The levels of MDA remained significantly unchanged in the MTX + CAPE group compared with the control group in the brain stem, spinal cord, and sciatic nerve of rats. As seen from the Tables, CAT activity in the spinal cord and sciatic nerve tissues increased significantly in the MTX group compared with the control group (p= 0.0001 for both). In the MTX + CAPE group, CAPE treatment significantly reduced the CAT activity in the spinal cord and sciatic nerve of rats, compared with the MTX group (p= 0.0001 for both). CAT activity remained significantly unchanged in the MTX + CAPE group compared with the control group in the spinal cord and sciatic nerve of rats (p> 0.05 for both).

The GSH-Px activity in the spinal cord and sciatic nerve increased significantly in the MTX group compared with the control group (p= 0.0001 for both). In the MTX + CAPE group, CAPE treatment significantly reduced the GSH-Px activity in the spinal cord and sciatic nerve of rats compared with the MTX group (p= 0.002 for both). However, GSH-Px activity was significantly higher in both the spinal cord and sciatic nerve tissues in the MTX + CAPE group than in the control group (p= 0.022, p= 0.016, respectively). The SOD activity was significantly higher in the MTX group than in the control group in the spinal cord and brain stem tissue (p= 0.001, p= 0.002, respectively). A significant decrease in SOD activity was found in the spinal cord and brain stem tissues when the MTX + CAPE group was compared with the MTX group (p= 0.004, p= 0.005, respectively). SOD activity in the spinal cord and brain stem tissues was not significantly different between the MTX + CAPE and the control groups (p> 0.05 for both). On the other hand, SOD activity in the sciatic nerve was not significantly different between groups (p> 0.05 for both).

Histopathological Results

The histopathological results are summarized in Table 4, and are shown in Figure 1. According to Table 4, increase in vascular proliferation and myelin degeneration and decrease in Schwann cells nuclear count, which were mentioned as findings of the sciatic nerve toxicity, were

Table 4. The comparison of Schwann cell nuclear count, degeneration in fibers and vascular proliferations in the sciatic nerve in the different groups

Groups	Number of Schwann cells nuclear count	Degeneration in myelinated fibers	Vascular proliferation
(I) Control (n= 6)	51.50 ± 3.56	0.33 ± 0.52	0.50 ± 0.54
(II) MTX (n= 6)	38.00 ± 2.19	2.83 ± 0.41	2.83 ± 0.41
(III) MTX + CAPE (n= 7)	47.00 ± 2.76	1.83 ± 0.75	1.67 ± 0.52
p values			
I-II	0.004	0.002	0.002
I-III	0.041	0.009	0.015
II-III	0.002	0.041	0.009

Values represent mean ± standard deviation. Statistical analysis was done using Mann-Whitney U test and $p < 0.05$ was accepted as statistically significant.

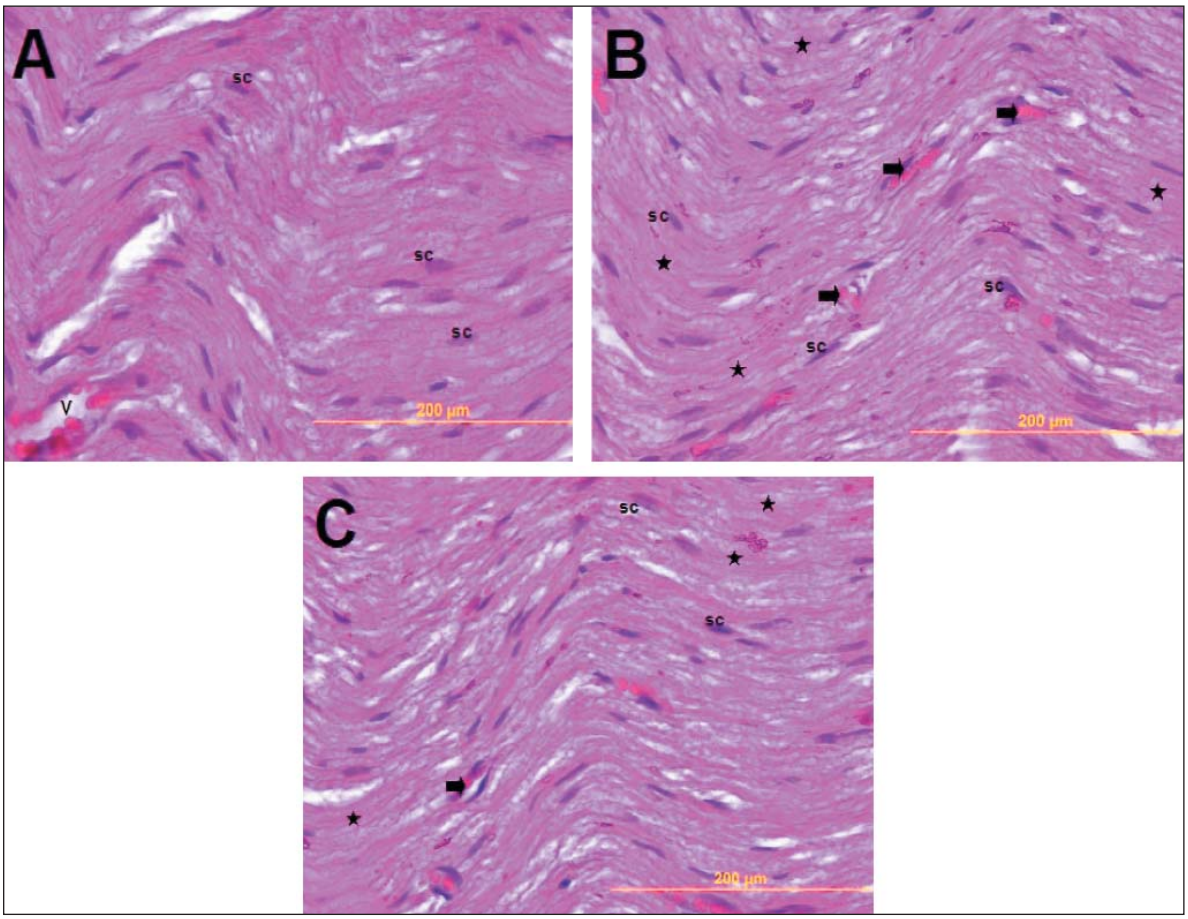

Figure 1. Sagittal sections histology of sciatic nerve in rats. **A.** Is normal appearance in control rat. V; vessel, sc; schwan cell (H&E, 200). **B.** Demonstrates increase vascular proliferation and myelin degeneration and decrease schwan cells in MTX administered rats. Black arrow; vascular proliferation, black star; myelin degeneration, sc; Schwan cell (H&E, 200). **C.** Demonstrates histological appearances of MTX plus CAPE administered rats Black arrow; vascular proliferation, Black star; myelin degeneration, sc; Schwan cell (H&E, 200).

significantly increased in the MTX-administered rats compared with the control rats (p values 0.002, 0.002 and 0.004, respectively). On the contrary, vascular proliferation and degeneration in myelinated fibers were decreased significantly in the MTX + CAPE administered rats compared with the MTX-administered rats (p values 0.009 and 0.041, respectively). The number of Schwann cells nuclear count was significantly increased in the MTX + CAPE

administered rats compared with the MTX-administered rats (p= 0.002).

DISCUSSION

Methotrexate is a potent anti-inflammatory and anticancer agent, which is widely used to control both neoplastic and arthritic processes (1-6). Nevertheless, a limiting factor in the use of MTX is its associated toxicity at multiple neuronal sites including the brain stem, spinal cord and peripheral nerve (3-8). Thus, shedding light on the mechanisms of action of MTX neurotoxicity may allow the development of compounds that can help to prevent the adverse effects of MTX.

Clinically, intrathecal MTX is usually used in the treatment of leukemia and other neoplasms infiltrating the CNS. However, transient or permanent neurological complications have been reported in a number of patients with or without CNS disease (8,9). It has been revealed that MTX induced dysfunction of the spinal cord and somatosensory pathways in a somatosensory evoked potential study (35). Although the adverse effect of MTX related to the CNS is well known, its adverse effect related to the PNS has not been studied previously. In this study, we aimed to first observe whether MTX caused neurotoxicity in the spinal cord, brain stem and sciatic nerve (PNS) of rats. Secondly, we assessed the MTX-induced neurotoxicity in the spinal cord, brain stem and sciatic nerve (PNS) of rats related to oxidative stress. The toxic dose of MTX has been shown in previous experimental studies. We also used a dose causing tissue toxicity (16,17). Many studies have been conducted to clarify the underlying mechanism of neurotoxicity caused by MTX (4,7,9,11). Miketova et al. found that MTX treatment applied to children with acute lymphoblastic leukemia caused oxidative stress in the CNS in a dose-dependent manner (9). In another study, Linnebank et al. showed that MTX-induced white matter changes are related to polymorphisms of methionine metabolism (4). MTX has been shown to lead to a depletion of methionine synthesis and to a lack of S-adenosyl-L-methionine (SAM) (acts as an antioxidant) in cerebrospinal fluid in patients with primary CNS lymphoma. (4,36). Because of the antioxidant effect of SAM, SAM deficiency caused by MTX may be a reason for the increased ROS (11,36). In addition, it has been shown that the adverse effect of MTX is partly due to the direct toxic effect by increasing ROS production (5). Babiak et al. suggested that the increase in oxidative stress was related to the effects of MTX with glutathione decreased (24). MTX-induced toxicity resulted in increased lipid peroxidation in different tissues of rats (16-18). We could not find any previous studies considering whether MTX induces oxidative stress in the brain stem and sciatic nerve tissues. Thus, the current study focused on the role of oxidative stress to clarify the underlying mechanism of MTX-induced neurotoxicity and to investigate whether or not CAPE has a protective effect on this toxicity.

In our study, MTX led to an increase in MDA levels, a reliable marker of lipid peroxidation in the brain stem, spinal cord and sciatic nerve. This finding shows that single dose IP administration of MTX results in oxidative stress in the brain stem, spinal cord and sciatic nerve of Wistar albino rats. Prophylactic CAPE treatment significantly ameliorated the increased MDA levels in the brain stem, spinal cord and sciatic nerve of rats. CAPE, serving as a free radical scavenger to inhibit peroxidation of membrane lipids, may maintain cell membrane integrity and function in the CNS and PNS of rats, thus contributing to its protective effects in CNS and PNS tissue (22,26,29). In several studies, it has been demonstrated that MTX-induced tissue damage (e.g., liver, kidney, gut tissue and cerebellum) could be prevented by some antioxidants such as melatonin, N-acetylcysteine and CAPE (7,16-18). In the present study, it was observed that CAPE significantly reduced the MTX-induced lipid peroxidation. With respect to the reduced oxidative damage due to CAPE treatment, many investigators have attributed the protective actions of CAPE to its antioxidative role, free radical scavenging effect, and neuroprotective and anti-inflammatory properties (12,22,26). It was reported that CAPE might protect the spinal and brain tissues from ischemia-reperfusion injury (23,37). It was also revealed that CAPE reduced lipid peroxidation in streptozotocin-induced diabetic rats and MTX-induced lipid peroxidation in cerebellum tissues (18,38). Hence, CAPE exerts a neuroprotective effect on the CNS against pentylenetetrazol-induced seizures in mice (12). They claimed that the neuroprotective effect of CAPE against neurotoxicity was associated with the blockade of nuclear factor κB, inhibition of caspase-1, caspase-3 and caspase-9 and inhibition of ROS (12,39,40). These reports are consistent with our findings that CAPE administration significantly afforded protection against oxidative stress induced by MTX.

In the brain stem and spinal cord tissues of rats, MTX treatment caused significant increase in SOD activity. The increased activity of SOD enzymes returned to nearly normal levels with CAPE administration. Antioxidants including the enzymatic system such as SOD, which converts superoxide anion to hydrogen peroxide (H_2O_2), provide the primary antioxidant defense (40,41). The increased enzyme activity of SOD in the MTX-administered group may be an adaptive response to the increased oxidative stress in MTX-induced neurotoxicity. This finding suggests an increase in the number of ROS in the brain stem and spinal cord tissue but not in the sciatic nerve. The eleva-

ted SOD activity is one of the most important components of the antioxidant defense system. The SOD enzyme protects cells against the toxic effect of superoxide radicals (40). The increased SOD activity may be another sign of increased oxidative stress in the neurotoxicity (18). Interestingly, statistically significant decreases in the SOD enzyme activity were found in the CAPE + MTX-administered group when compared to the MTX group. CAPE might be a scavenger of ROS such as superoxide radicals (26). For this reason, CAPE may prevent the elevation in the SOD activities by MTX in the brain stem and spinal cord. While we did not observe a significant change in the SOD activity in sciatic nerve tissue, MTX did cause a significant increase in SOD activity in the brain stem and spinal cord tissues of rats.

Glutathione peroxidase and CAT enzymes are important antioxidant enzymes that play a role in elimination of hydrogen peroxide and lipid hydroperoxides and decrease peroxides by using reduced glutathione as a reactive hydrogen radical donor (41). In this study, GSH-Px and CAT activities in sciatic and spinal cord tissues were increased significantly in MTX-treated rats compared to the control rats. The increased GSH-Px and CAT enzyme activities reflect the increased production of hydrogen peroxide or increased antioxidant enzyme activities and are considered to be the protective response of the living body against increased oxidative stress by ROS (42). In the MTX + CAPE group, CAPE treatment significantly decreased GSH-Px and CAT levels. CAPE might be a scavenger for free oxygen radicals, which in turn may avert the elevation in GSH-Px and CAT activities. Increased GSH-Px and CAT activities may be other signs of the increased oxidative stress in MTX-induced neurotoxicity. In the present study, a statistically significant decrease in the number of Schwann cells was found in the MTX group compared to the control group.

The number of Schwann cells increased in the MTX + CAPE group when compared with the MTX-administered group. Otherwise, vascular proliferation and degeneration in myelinated fibers significantly decreased in MTX + CAPE-administered rats compared with the MTX-administered rats. The number of Schwann cells nuclear count significantly decreased in the MTX-administered rats compared with the control rats. Because of the neuroprotective effect of CAPE, Schwann cells nuclear count was significantly higher in the MTX + CAPE group than in the MTX-only treatment group. This histopathological result demonstrated that MTX caused peripheral nerve toxicity and that CAPE decreased this toxicity.

This experimental study reveals important findings relating to oxidative stress in MTX-induced neurotoxicity in the sciatic nerve, spinal cord and brain stem of rats. Firstly, we demonstrated that MTX treatment causes oxidative damage biochemically by increasing the levels of MDA and antioxidant enzyme activities in neuronal tissues of rat. Secondly, co-treatment with the antioxidant CAPE, a potent free radical scavenger agent, significantly prevented oxidant damage in the MTX-induced neurotoxicity.

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