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Effect of Caffeic Acid Phenethyl Ester on Cerebellar Tissue Damage Secondary to Methanol Intoxication: Experimental Study

Metonol İntoksikasyonuna Bağlı Serebeller Doku Hasarı Üzerine Kafeik Asit Fenetil Esterin Etkisi: Deneysel Çalışma

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Summary

Objective: Previous studies have shown the role of oxidative stress in methanol neurotoxicity. CAPE is known to have an antioxidant property that is shown in many experimental studies. In this study, we aimed to investigate whether CAPE has a protective effect against oxidative stress observed in the cerebellar tissue in methanol intoxication.

Material and Method: In this study, a total of 40 rats were split into 5 groups: control group (n=8), MTX-alone group (n=8), MTX+methanol group (n=8), MTX+methanol+ethanol group (ethanol group) (n=8), and MTX+metanol+CAPE group (CAPE group) (n=8). All the rats except the control group were delivered methotrexate (MTX) therapy (0.3 mg/kg/day, via i.p. route) for 7 days in order to induce methanol toxicity. The control group received no drug therapy. Seven days later, 3 g/kg (i.p.) methanol was delivered in the ethanol and CAPE groups. Four hours after the delivery of methanol, ethanol group received 0.5 g/kg ethanol (i.p.) and CAPE group received 10 µmol/kg CAPE (i.p.), while the other groups were delivered only saline (i.p.). The rats were decapitated after 8 hours and the cerebellar tissues were removed. PON-1, TAS, and MDA levels were measured in the tissues.

Results: MTX-alone group demonstrated decreased TAS and PON-1 levels (p=0.001 and p=0.004, respectively) and increased MDA level (p=0.001), as compared to the control group. When MTX+methanol group was compared with the MTX-alone group, MTX+methanol group was found to have decreased TAS and PON-1 activities (p=0.037 and p=0.046, respectively) and increased MDA level (p=0.022). The ethanol group was found to show a significant decrease in MDA level (p=0.001), as compared with the MTX+methanol group. The CAPE group exhibited increased TAS and PON-1 levels (p=0.001 and p=0.001, respectively) and decreased MDA levels, as compared with the MTX+methanol group.

Discussion: Cerebellum demonstrates oxidative stress secondary to methanol intoxication. CAPE therapy is more effective against cerebellar oxidative stress than ethanol therapy. *(Turkish Journal of Neurology 2013; 19:93-6)*

Key Words: Cerebellum, oxidative stress, methanol, ethanol, CAPE

Özet

Amaç: Metanol nörotoksisitesinde oksidatif stresin rolü olduğu daha önceki çalışmalarda bildirilmiştir. Kafeik asit fenetil esterin (CAPE) birçok deneysel çalışmada antioksidan özelliği gösterilmiştir. Bu çalışmada amacımız ratların serebeller dokusunda metanol toksisitesine karşı ortaya çıkan oksidatif strese karşı CAPE'nin koruyucu etkisi olup olmadığını araştırmaktır.

Gereç ve Yöntem: Bu çalışmada 40 adet rat; kontrol (n=8), yalnız metotreksat (MTX) verilen (n=8), MTX+metanol (n=8), MTX+metanol+etanol tedavisi (etanol grubu) (n=8), ve MTX+metanol+CAPE tedavisi (CAPE grubu) (n=8), olmak üzere 5 ayrı gruba ayrıldı. Kontrol grubu dışındaki tüm ratlara metanol toksisitesi oluşturabilmek için 7 gün boyunca MTX tedavisi (0,3 mg/kg/gün intraperitoneal (i.p.)) verildi. Kontrol grubuna herhangi bir ilaç verilmedi. 7 gün sonra metanol 3 g/kg (i.p.) dozunda metanol, etanol ve CAPE gruplarına verildi. Metanol verildikten 4 saat sonra etanol tedavi grubuna etanol 0,5 g/kg (i.p.), CAPE tedavi grubuna 10 µmol/kg CAPE (i.p.), diğer gruplara serum fizyolojik (i.p.) verildi. 8 saat sonra ratlar dekapite edilerek serebellum dokuları çıkarıldı. Dokularda paroksanaz-1 (PON-1), total antioksidan kapasite (TAS) ve malondialdehit (MDA) düzeyleri ölçüldü.

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Bulgular: Yanlız MTX alan grupta kontrol grubuna göre TAS ve PON-1 düzeyinde azalma (sırasıyla p=0,001, p=0,004) ve MDA düzeyinde artış (p=0,001) saptandı. MTX+metanol grubu yalnız MTX alan grupla karşılaştırıldığında MTX+metanol grubunda TAS ve PON-1 aktivitesinde azalma (sırasıyla p=0,037;p=0,046) ve MDA düzeyinde ise artış (p=0,022) saptandı. Etanol tedavi grubu MTX+metanol grubuyla karşılaştırıldığında etanol tedavi grubunda MDA düzeyinde anlamlı düzeyde azalma saptandı (p=0,001). CAPE grubu MTX+metanol grubuyla karşılaştırıldığında CAPE tedavi grubunda TAS ve PON düzeyinde artış (sırasıyla p=0,001, p=0,001) ve MDA düzeyinde azalma (p=0,001) bulundu.

Sonuç: Metanol intoksikasyonuna bağlı serebellumda oksidatif stres ortaya çıkmaktadır. CAPE tedavisi serebellumda ortaya çıkan oksidatif stresi etanol tedavisine göre daha fazla azaltmaktadır. *(Türk Nöroloji Dergisi 2013; 19:93-6)*

Anahtar Kelimeler: Memantin, apopitoz, iskemik inme, antioksidan, oksidan

Introduction

Methanol is a colorless, odorless and toxic type of alcohol that is commonly used in the industrial production of a variety of synthetic organic compounds (1). Methanol intoxication may occur as the result of an accident, due to the consumption of bootleg alcoholic beverages or as suicide attempts (2). The clinical variety of the toxic symptoms includes headaches, dizziness, visual impairments, cerebral infarcts, coma following cerebral edema and death. Even though methanol itself is not severely toxic, alcohol-dehydrogenase metabolizes it to extremely toxic formaldehyde and later on to formic acid. Formic acid causes a reduction in ATP synthesis, an increase in reactive oxygen species (ROS) and cell deaths either directly or through the inhibition of cytochrome oxidase in the mitochondrial respiratory chain (3). The most important product of the ROS increase in the brain during the acute stage is the malondialdehyde (MDA) resulting from the lipid peroxidation. Malondialdehyde is a parameter used to indicate oxidative stress. Paroxanase-1 (PON-1) is an enzyme that plays an antioxidant role against cellular damage by hydolizing lipid peroxides. Total antioxidant status (TAS) measurement is a more reliable antioxidant parameter since it reflects the total antioxidant capacity instead of individual measurements of antioxidant matters and enzymes in the plasma (4). Previous studies reported the role of oxidative stress in methanol neurotoxicity. Farbiszewski et al. showed the toxic effect of methanol in rat brain and the reduction of antioxidant capacity (5). In the autopsies of methanol intoxication cases, degeneration was found in the cerebellar granular cell layer (6). In addition, experimental studies showed an increase in cerebellar ROS due to methanol intoxication and a reduction in antioxidant capacity (7). For this purpose, we reasoned that antioxidant treatments might play a preventative role against this toxicity.

Cafeic acid phenethyl esterin (CAPE) is an active component of flavonoid structure. In addition to its antioxidant effect, it has many other biological and pharmacological properties such as being anti-inflammatory, anti-carcinogenic, antiviral, neuroprotective and immunomodulating (8). In methanol toxicity, ethanol and fomepizole (alcohol dehydrogenase inhibitor) are used in order to prevent the conversion of methanol to toxic metabolites. In the recent years, experimental studies also used substances that are shown to reduce the metabolic acidosis and oxidative stress due to methanol toxicity (9, 10). To our knowledge, the protective effect of CAPE against methanol toxicity is currently not known. In this study, we aimed to investigate whether CAPE has a protective effect against oxidative stress observed in the cerebellar tissue of rats during methanol intoxication.

Materials and Methods

Chemicals

Methotrexate was supplied from Koçtaş-İstanbul and dissolved in normal saline solution. Methanol, ethanol and CAPE were supplied from Sigma Chemical firm. Methanol and ethanol was dissolved in normal saline solution and applied as 20% v/v solution. CAPE was dissolved in normal saline solution and dimethyl sulfoxide.

Rats and Treatment

The study was conducted in Dicle University Faculty of Medicine Experimental Animal Laboratory after being approved by Dicle University Experimental Animals Ethical Board. In the study, 40 Sprague-Dawley rats (320±20 g) were used. Rats were kept in an environment with standard lighting (12 hours daylight/12 hours of darkness) and temperature (22±2°C) in plastic cages with adequate food and water. Compared to humans, folate content in rat liver is higher and their folate metabolism is faster. For this reason, inducing formic acid accumulation and metabolic acidosis is difficult. In experimental studies, it was shown that methotrexate (MTX) reduces folate content in rats (7). Therefore, in order to induce a methanol intoxication in rats that is comparable to humans, rats were given a 7-day-log MTX treatment (0.3 mg/kg/day intraperitoneally [IP]) to reduce their folate metabolism. Rats were divided in to 5 groups as controls (n=8), only MTX group (n=8), MTX+methanol group (n=8), MTX+methanol+ethanol treatment (n=8) (ethanol group), and MTX+methanol+CAPE treatment (n=8) (CAPE treatment). Except for the control group, all of the rats received MTX as IP. Control group did not receive any treatment. After 7 days, 3 g/kg (IP) methanol was administered to methanol, ethanol and CAPE groups. Four hours after the methanol administration, ethanol group received 0.5 g/kg (IP) ethanol, CAPE group received 10 µmol/kg CAPE (IP), and the remaining groups received saline solution (IP). After 8 hours, rats were anesthetized by administering 50 mg/kg ketamine HCL. Following the anesthesia, rats were decapitated and their cerebellar tissues were carefully removed. The tissues acquired for the subsequent biochemical analyses were evaluated for their PON-1, TAS and MDA levels.

Biochemical Measurements

The cerebellar tissue samples acquired for the biochemical measurements were washed in cold (+4°C), 0.15M potassium chloride (KCl) and dried with drying paper. After that, the tissues were homogenized inside an ice container with the same solution at 1/5 (w/v) ratio. The measurements were taken from the supernatant volume acquired after 30 minutes of centrifuge at 10000rpm. The homogenization was done inside an ice container.

Table 1. PON-1, TAS and MDA levels in cerebellar tissues of rats

Groups	PON-1 activity (U/mg protein)	TAS (mmolTroloxEq./g protein)	MDA(nmol/g protein
Control (I) (n=8)	3.73±1.42	0.40±0.06	16.85±3.56
Only MTX (II) (n=8)	2.10±0.44	0.30±0.10	26.76±6.66
Methanol+MTX (III) (n=8)	1.27±0.33	0.23±0.49	32.80±5.26
Methanol+MTX+Ethanol tx.(IV) (n=8)	0.73±0.21	0.30±0.56	23.05±3.68
Metanol+MTX +CAPE tx. (V) (n=8)	2.98±0.79	0.38±0.57	21.32±5.43
Groups		**p value**	
I-II	0.001	0.004	0.001
II-III	0.037	0.046	0.022
III-IV	0.17	0.063	0.001
III-V	0.001	0.001	0.001
IV-V	0.001	0.02	0.49
I-IV	0.001	0.003	0.019
I-V	0.061	0.44	0.086

MDA: Malondialdehyde. TAS: Total Antioxidant Status. PON-1: Paroxanase-1:

The homogenates were kept at -40 °C until the analysis time (1 week). Tissue MDA levels were measured with Ohkawa method (11), paraoxonase enzyme activity was measured with Eckerson method (12) and TAS was measured using the photometric technique developed by Erel et al. (4). Tissue protein levels were measured with Lowry method (13).

Statistical Analyses

SPSS (Statistical Package for Social Sciences) 14.0 was used for the statistical analyses of the data in the study. The results were expressed as average±standard deviation. One-way ANOVA was used to evaluate the group differences in biochemical values, following LSD (Least significant difference) as the post-hoc test. A statistical threshold of $p<0.05$ was used for statistical significance.

Results

The TAS, PON-1 and MDA levels in the cerebellar tissue of the rats are shown in Table 1. The group that took only MTX had significantly lower levels of TAS and PON-1 (p=0.001 and p=0.004 respectively) and higher levels of MDA (p=0.001) as compared to the control group. When CAPE group was compared to the control group, TAS and PON-1 activity was found to be decreased and MDA was found to be increased, but these differences failed to meet statistical significance. When ethanol group was compared to the control group, TAS and PON-1 activity decreased significantly (p=0.001 and p=0.003 respectively) and MDA increased significantly (p=0.019). When MTX+methanol group was compared to only MTX group, MTX+methanol group was seen to be significantly low in TAS and PON-1 activity (p=0.037 and p=0.046 respectively) and MDA was seen to be significantly high (p=0.022). When ethanol treatment group was compared to the MTX+ethanol group, PON-1 was seen to have decreased insignificantly and TAS was seen to have increased also insignificantly, while the decrease in MDA level was significant (p=0.001). When CAPE group was compared to MTX+methanol group, CAPE treatment was seen to have caused significant increases in TAS and PON-1 levels (p=0.001 and p=0.001 respectively) and a significant decrease in MDA level (p=0.001). When CAPE group was compared to ethanol group, there was an increase in PON-1 and TAS levels (p=0.001 and p=0.02 respectively) and an insignificant decrease in MDA levels.

Discussion

The results of the present study suggest that methanol toxicity caused oxidative stress in cerebellar tissue in rats, and CAPE and ethanol treatments reduced the oxidative stress caused by methanol toxicity. The oxidative stress due to methanol toxicity was reduced more effectively by CAPE treatment compared to ethanol treatment. Rajamani et al. evaluated the oxidative stress at different sites on the brain using only MTX and MTX+methanol. Their findings suggested that the oxidative stress was higher in MTX+methanol group compared to the group that was given only MTX (7). In our study, we found that PONS and TAS levels were higher and MDA level was higher in MTX+methanol group compared to the group that was given only MTX. Since the substance responsible for methanol toxicity is formic acid, the treatment depends on the inhibition of alcohol dehydrogenase enzyme, which is the first step of conversion to formic acid. For this purpose, the contemporary practice is to use ethanol and fomepizole which have higher affinities to alcohol dehydrogenase enzyme (14). Ethanol reduces the metabolic acidosis due to methanol toxicity but a recent experimental study showed that ethanol exposure also leads to neuronal damage by increasing oxidative stress (15).

Furthermore, it was shown that acute exposure to ethanol stimulates GABA neurotransmitters in cerebellar Golgi cells (16). Therefore, there has been a recent effort to investigate the substances

alternative to the use of ethanol in methyl alcohol intoxication. El-Bakary et al. showed in an experimental study that Ranitidine alleviates the metabolic acidosis due to methanol toxicity and restores retinal damage by causing alcohol dehydrogenase inhibition (9). Another study showed the protective effects of melatonin on liver damage due to methanol toxication (10). In our study, the TAS levels, which are the indicators of total antioxidant levels in the plasma was increased in statistically insignificant amounts in the ethanol group as compared to the MTX+ethanol group, even though we found a statistically insignificant decrease in PON-1 activity. The MDA level, which is an indicator of the oxidative stress also increased significantly in the ethanol treatment group. In other words, our results showed that ethanol reduces the oxidative stress due to methanol toxicity. However, we found increases in both PON-1 and TAS levels and a decrease in MDA levels in the group that received CAPE treatment as compared to MTX+methanol group. This shows that the oxidative stress was decreased more in CAPE group than ethanol treatment group. CAPE's protective effects stemming from its antioxidant and antiproliferative properties have been shown in brain ischemia reperfusion and spinal ischemia reperfusion damage, multiple sclerosis and convulsions (17,18,19,20). Moreover, a study on alcohol-fed rats showed a decrease in lipid peroxidation products upon administration of cafeic acid, which is CAPE's metabolite (21,22). To our knowledge, our study shows the effect of CAPE treatment on reduction of oxidative stress due to methanol toxicity for the first time in literature.

In conclusion, methanol intoxication causes oxidative stress in cerebellum. In this study, we shoved that CAPE treatment is more effective in reducing the cerebellar oxidative stress compared to ethanol treatment. These findings may indicate CAPE as an alternative treatment option in methanol intoxication.

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