DIETARY ADDITION OF CAFFEIC ACID PHENETHYL ESTER PROTECTS MYOCARDIAL TISSUE AGAINST ETHAMBUTOL INDUCED OXIDATIVE STRESS

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ABSTRACT

Objective: *The myocardial effect of ethambutol (ETM) has not yet been clarified. The main purpose of this study was to determine both the oxidative status in myocardial tissue after administration of ETM and the adjuvant benefits of caffeic acid phenethyl ester (CAPE).*

Material and Method: *Twenty four male rats were divided into three experimental groups as follows: a control group (without any drug administration) was created for obtaining normal myocardial tissue; an ETM group (rats received only ETM for thirty days) was created for ethambutol administration; and an ETM+CAPE group was created for administration of the full regimen (rats received ETM+CAPE for thirty days). Rats were sacrified at the end of day 30 and heart tissues were obtained for histopathological and biochemical examination. Oxidant and antioxidant parameters were biochemically investigated in all tissue samples.*

Results: *In the ETM group, myocardial malondialdehyde (MDA) levels and total oxidant status (TOS) were significantly higher than in the control group ($p<0.001$). Conversely, total antioxidant capacity (TAC), the activity of superoxide dismutase (SOD) and of serum paraoxonase (PON1) were reduced in the ETM group ($p<0.05$).*

Furthermore, MDA and TOS activity was significantly reduced in the ETM+CAPE group ($p<0.05$); TAC, SOD, and PON1 activities were increased with adjuvant CAPE therapy (in the ETM+CAPE group) rather than in the ETM group.

Conclusion: *ETM may lead to increased myocardial oxidative stress due to lipid peroxidation. Nevertheless, adjuvant CAPE administration seems to provide a partial enhancement of myocardial damage*

Key Words: *CAPE compound, ethambutol, myocardium, oxidative stress* ***Nobel Med 2013; 9(3): 120-124***

KAFEİK ASİT FENETİL ESTERİN DİYETE EKLENMESİ ETAMBUTOL KAYNAKLI OKSİDATİF STRESE KARŞI TEDAVİSİNİN MİYOKARD DOKUSUNU KORUR

ÖZET

Amaç: Etambutolün (ETM) miyokardiyal etkileri henüz aydınlatılmamıştır. Bu çalışmada hem ETM uygulanmasından sonra miyokardiyal dokudaki oksidatif durumu hem de kafeik asit fenetil ester (KAPE)'in adjuvan faydalarının belirlenmesi amaçlanmıştır.

Materyal ve Metod: Yirmi dört erkek rat, normal miyokardiyal doku eldesi için kontrol grubu (herhangi bir uygulama yapılmamış), etambutol uygulanmış ETM grubu (ratlara 30 gün boyunca sadece ETM verilmiştir) ve tam tedavi rejimi verilen ETM+KAPE grubu (ratlara 30 gün boyuna ETM + KAPE verilmiştir) olmak üzere 3 deneysel gruba bölünmüştür. Otuz günün sonunda ratlar sakrifiye edilerek histopatolojik ve biyokimyasal analiz için kalp dokuları alınmıştır. Biyokimyasal olarak, tüm doku örneklerinden oksidan ve antioksidan parametreler araştırılmıştır.

Bulgular: ETM grubunda, miyokardial malondialdehit (MDA) seviyeleri ve total oksidan seviyeleri (TOS) kontrol grubuna oranla anlamlı derecede yüksekti ($p<0,001$). Tersine, toplam antioksidan kapasite (TAK), süperoksit dismutaz (SOD) ve serum paraoksonaz (PON1) aktivitesini ETM grubunda ($p<0,05$) azalmıştı. Ayrıca, MDA ve TOS aktivite önemli ölçüde ETM + KAPE grubunda ($p<0,05$) azalmıştı; TAC, SOD, ve PON1 aktiviteleri ise ETM grubuna göre adjuvan KAPE tedavisi verilen grupta (ETM + KAPE grubunda) artmıştı.

Sonuç: ETM miyokardiyal dokuda lipid peroksidasyonu ile oksidatif streste artışa yol açabilir. Bununla birlikte, adjuvan KAPE uygulanması miyokardiyal hasarda kısmi iyileşme sağlıyor gibi görünmektedir.

Anahtar Kelimeler: KAPE bileşiği, etambutol, miyokard, oksidatif stres. **Nobel Med 2013; 9(3): 120-124**

INTRODUCTION

Ethambutol dihydrochloride (ETM) is an important agent for the initial treatment of tuberculosis. ETM is a dextro form of 2,2'-ethylene-diiminol-di-1-butanol dihydrochloride that is rapidly and widely distributed into most body tissues and fluids after administration. It does not disrupt the structure of mycolic acid, but rather is inhibited to participation of the cellular wall. Ethambutol binds intracellular zinc and copper through chelation, which helps explain the antituberculous and toxic efficacy of ETM.[1-3] To date, there have been some studies that investigated the myocardial effects of ETM.[4,5] However, no exact data exists related to the myocardial oxidative effects of ETM.

Preventive techniques have been improved to avoid the irreversible impact of free oxygen radicals on exposed tissues during use of certain drug therapies. Therefore, combination or adjuvant therapies are being used more often in clinical practice.[6,7] Caffeic acid phenethyl ester (CAPE) is one of the current agents for combination antioxidant therapies. Caffeic acid phenethyl ester is an active component of propolis and has a structure similar to that of flavonoids. It inhibits oxygenation of linolenic and arachidonic acid that is catalyzed by 5'-lipoxygenase in micromolar concentrations. Many studies have examined the effectiveness of CAPE in the context of its ability to help prevent oxidative stress caused by use of drugs such as isoniazid.[6-8] In the present study, ETM was investigated in terms of its potential to contribute to myocardial edema and oxidative reactions. Additionally, the potential beneficial role of the frequently used antioxidant substance CAPE was researched.

MATERIAL and METHOD

Study Design

This study was a randomised, controlled, single-blinded, interventional animal study. The study protocol was approved by the Local Animal Ethics Committee and conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Local Animal Ethics Committee (Ethical Committee of Dicle University, 31.05.2010-21).

Animal Subjects

Twenty-four male Sprague-Dawley rats (aged 8-12 weeks) weighing 230±30 g (mean±standard deviation) obtained from the Laboratory Animal Production Unit were used in the experiment. The rats were placed in a room controlled for temperature (22±2°C) and humidity (50±5%) with a 12-hour light/dark cycle for 1 week before the experiment was initiated. A standard diet and tap water were provided ad libitum. The rats were given only water for 12 hours before the experimental procedure was initiated.

Study Protocol and Tissue Sampling

Four of the rats were used for creation of the control group. The remaining 20 rats were randomised into 2 different groups of 10 animals each as follows: →

Table 1: Comparison of oxidative markers between the control and ETM groups

	TAC mmol Trolox Eq/g protein	TOS mmol H_2O_2 Eq/g protein	MDA nmol/ml	SOD U/ml/g	PON^{-1} U/L
Control	0.52±0.11	155.5±41.2	236.0±23.3	3.88±0.57	16.1±1.3
ETM	0.36±0.1	234.7±70.9	360.5±47.8	2.14±1.12	9.9±1.9
***p**	0.03	0.001	0.001	0.001	0.001

TAC: Total antioxidant capacity; TOS: Total oxidant status; MDA: Malondialdehyde; SOD: Superoxide dismutase; PON^{-1}: Serum paraoxonase; ETM: Ethambutol. *p<0.05 is significant.

Table 2: Comparison of oxidative markers between ETM and ETM+CAPE groups

	TAC mmol Trolox Eq/g protein	TOS mmol H_2O_2 Eq/g protein	MDA nmol/ml	SOD U/ml/g	PON^{-1} U/L
ETM	0.36±0.1	234.7±70.9	360.5±47.8	2.14±1.12	9.9±1.9
ETM+CAPE	0.55±0.17	160.2±22.7	239.1±44.3	3.01±1.2	13.1±1.7
***p**	0.001	0.001	0.001	0.091	0.033

TAC: Total antioxidant capacity; TOS: Total oxidant status; MDA: Malondialdehyde; SOD: Superoxide dismutase; PON^{-1}: Serum paraoxonase; ETM: Ethambutol; ETM+CAPE: Ethambutol+Caffeic acid phenethyl ester. *p<0.05 is significant.

- ETM-administered group (ETM group): ETM was administered through tap water (50 mg/kg/day) for 30 days via gavage.
- ETM-administered with additive CAPE group (ETM+CAPE): ETM was administered in combination with CAPE through tap water (50 mg/kg/day) for 30 days via gavage.[7]

Rats were sacrified with ethylic ether inhalation, and then cardiac tissue samples were obtained for histopathological examination.

Biochemical Analysis

The cardiac tissue samples were stored at -50ºC until examination. The protein concentration of tissues was evaluated after centrifugation at 14000 rpm (~20000 x g) for 30 minutes at 4ºC for homogenisation as previously described by Lowry.[9] Superoxide dismutase (SOD) activity was evaluated using the Fridovich Method.[10] Serum paraoxonase (PON1) activity was evaluated with a modified Eckerson Method.[11] Cardiac lipid peroxide levels were expressed as malondialdehyde (MDA) levels, which were obtained using a procedure previously described by Ohkawa et al.[12] The total oxidant status of plasma was measured using a novel automated colorimetric measurement method for total oxidant status (TOS) developed by Erel.[13] The assay was based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured with spectrophotometry, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/l). Total antioxidant capacity of plasma was measured using a novel automated colorimetric measurement method for total antioxidant capacity (TAC) developed by Erel.[14] In this method, hydroxyl radical was produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which was bright yellowish-brown in color. After addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measurement of TAC. The assay results were expressed as mmol Trolox Eq/l.[15]

Histopathological Analysis

Myocardial tissue specimens were fixed in 10% formalin, routinely processed, and embedded in paraffin. Paraffin sections for light microscopy were typically 4 μm thick and stained with hematoxylin and eosin (H&E) for examination under a light microscope (Nikon Eclipse 80i, Japan) by an expert histologist.

Statistical Analysis

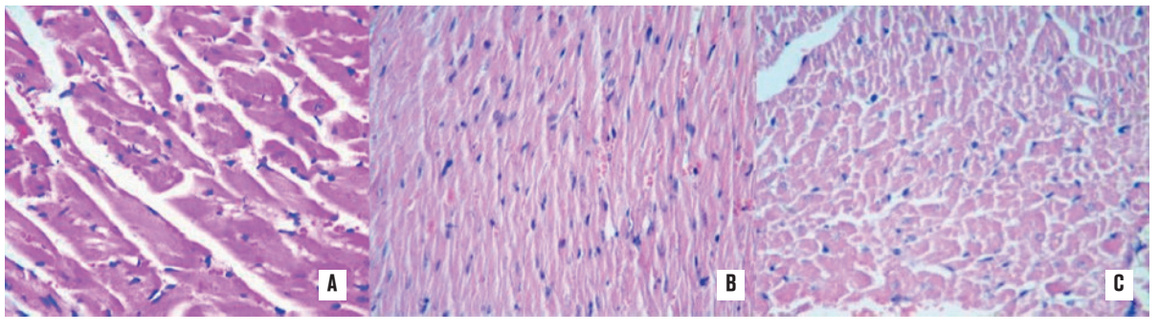
Grouped data were presented as means±standard deviations. The Kolmogorov-Smirnov test was used to evaluate normalisation distribution for each variable. The Mann-Whitney U test was used for nonparametric variables.

RESULTS

The obtained baseline values in the control group were as follows: TAC=0.52±0.11 mmol Trolox Eq/g protein; TOS=155.5±41.2 mmol H_2O_2 Eq/g protein; MDA=236.0±23.3 nmol/ml; SOD=3.88±0.57 U/ml/g; PON1=16.1±1.3 U/L.

In the ETM group, MDA and TOS levels were determined as 360.5±47.8 nmol/ml and 234.7±70.9 mmol H_2O_2 Eq/g protein, respectively. Both of these values were higher than those in the control group (p=0.001). Conversely, MDA and TOS levels were lower in the ETM+CAPEETM+CAPE group (MDA=239.1±44.3 nmol/ml; TOS=160.2±22.7 mmol H_2O_2 Eq/g protein), almost matching the values from the control group. The differences between ETM and ETM+CAPE groups according to MDA and TOS levels were statistically significant (*p*=0.001).

In the ETM group, values for activity of TAC (0.36±0.1 mmol Trolox Eq/g protein), SOD (2.14±1.12 U/ml/g), and PON1 (9.9±1.9 U/L) were lower than those for →



Şekil 1a: (A) Normal myocardial morphology in the control group. (H&E, 400x)*; (B) Partially injured myocardium characterised by hydropic cardiomyocytes in the ETM+CAPE group (H&E, 400x); (C) Advanced injury in myocardium characterised by predominant contraction bands in the field of view (including severe edema) in the ETM group.
* H&E, 400x: Hematoxylin and eosin stain, magnification at 400x.

the control group (p<0.05). In the ETM+CAPE group, the values for PON-1 (13.1±1.7 U/L), TAC (0.55±0.17 mmol Trolox Eq/g protein), and SOD (3.01±1.2 U/ml/g) were higher than in ETM group. With the exception of the value for SOD activity (p<0.091), all of these differences were statistically significant (p<0.05). The comparisons of the oxidative markers between each group are summarized below in Tables 1 and 2.

Advanced oedema was detected on microscopic examination in the ETM group (Figure 1C) according to baseline characteristics in the control group (Figure 1A). Moreover, remarkable lower myocardial oedema was detected in the ETM+CAPE group (Figure 1B).

DISCUSSION

In a healthy body, an important balance is maintained between the oxidant and antioxidant systems; cellular mechanisms are managed in order to avoid the toxic factors in oxidative stress conditions. When the correct balance breaks down, a sequence of adverse events begins with edematous cellular swelling, resulting in cellular functional loss.[16] Ultimately, the redox reactions may lead to atherosclerosis, myocardial oedema, and endothelial dysfunction in the cardiovascular system.[16,17] Meanwhile, pharmaceutical therapies may cause apoptosis by triggering oxidative reactions.[18] Measurements of malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC), superoxide dismutase (SOD), and serum paraoxonase (PON1) activities are important markers of oxidative stress and reactions.[19-22]

In the current study, the myocardial oxidative effects of ETM and the preventive effects of CAPE reinforcement were investigated through measurement of oxidative stress markers and histopathological examination.

ETM has a lower affinity for plasma proteins, but can easily penetrate erythrocytes and can store. The adverse events associated with ETM are usually neural, dermal, or hepatic; the most frequently reported toxic side effect is optic neuritis, which may be persistent in some cases.[23,24] Cardiac effects have been rarely reported with ETM, and the available reports in the literature are dated. In 1965, Cappiello et al. reported an experimental study involving ETM administration in dogs.[5] They claimed that the deaths reported in the study were related to cardiac failure after ETM administration. [5] Nevertheless, according to Physician's Desk Reference data reported by Ordell in 1989 (from animal toxicology studies designed with dogs), prolonged administration of ETM in large doses may cause myocardial damage, the development of heart failure, and depigmentation of the tapetum lucidum.[25] In contrast, Kelly et al. reported that distributions of ETM were high in the liver, kidney, and lung, but that the heart was not affected to the same extent as were these other organs.[4]

Caffeic acid phenethyl ester (CAPE) is a biologically active component of honeybee propolis that forms like flavonoids and can inhibit the oxidative reactions induced due to interactions between 5'-lipoxygenase and linolenic acid-arachidonic acid.[8] Caffeic acid phenethyl ester is currently a popular agent, with features such as anti-free radical, anti-oxidant, and anti-inflammatory activities.[26] Akyol et al. have stated that CAPE might have a beneficial impact in palliating side effects related to chemotherapy and radiotherapy.[27]

In the present study, increased levels of MDA and TOS were obtained in the ETM group; lower activity levels for TAC, SOD, and PON1 were also detected in comparison with the baseline levels in the control group (p<0.05). Reduced levels of MDA, TOS, and increased activity levels for PON1, SOD, and TAC were obtained in the ETM+CAPE group. The difference between ETM and ETM+CAPE groups according to these factors was statistically significant (p<0.05). SOD levels were also increased in the ETM+CAPE group, but this difference was statistically insignificant (p=0.091). During histopathological examination, marked myocardial →

oedema was present in the ETM group (Figure 1B), but remarkable lower myocardial oedema was detected in the ETM+CAPE group (Figure 1C). Namely, oxidation markers and myocardial oedema were lower in the ETM+CAPE group.

CONCLUSION

Our results support the hypothesis that ETM may have oxidant effects on myocardial tissue, and that supplementation with CAPE may prevent this oxidative damage. ETM may act as an oxidative trigger, with storage in erythrocytes and release during oxygen delivery. CAPE may have reliable cardioprotective effects against various damaging agents, including free radicals. We strongly suggest that further studies should be performed to identify the myocardial impact of ETM and clarify the cardioprotective effects of CAPE.

Declaration of conflicting interests

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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DELIVERING DATE: 26 / 10 / 2012 • **ACCEPTED DATE:** 29 / 03 / 2013

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