

Effects of Epigallocatechin 3- Gallate in Rat Cardiac Tissue on Oxidant and Antioxidant System Exposed to Sevoflurane Anesthesia

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ABSTRACT

Objectives: To investigate the possible effects of sevoflurane anesthesia and epigallocatechin 3- gallate (EGCg) on cardiac tissue by evaluating the oxidant and antioxidant status in rats.

Materials and Methods: Three groups of animals were studied. Sevoflurane 3% (v/v) in air/O₂ were administered to animals in group 1 (n = 6) and sevoflurane plus EGCg was administered in group 2 (n = 6). Six animals were allocated to control group (group 3). Malondialdehyde (MDA), nitric oxide (NOx), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in cardiac tissue were studied.

Results: In group 1, MDA, SOD and GSH-Px levels were significantly increased (p<0.05, p<0.0001, p<0.001, respectively) while non-significant changes occurred in CAT and NOx activities. Supplementation of EGCg (group 2) decreased the MDA, SOD and GSH-Px activities whereas, the NOx activities increase with EGCg supplementation.

Conclusion: The amount of lipid peroxidation and antioxidant enzyme levels were more increased in following sevoflurane administration. The administration of intravenous EGCg significantly protected cardiac tissue. ©2007, Fırat University, Medical Faculty

Key words: EGCg, sevoflurane, oxidant-antioxidant system

ÖZET

Sevofluran Anestezisi Uygulanmış Rat Kalb Dokularında Oksidan ve Antioksidan Sistem Üzerine Epigallocatechin 3- Gallate'in Etkileri

Amaç: Oksidan ve antioksidan durumun değerlendirilmesi ile kardiyak doku üzerine sevofluran anestezisi ve epigallocatechin 3- gallate (EGCg)'nin muhtemel etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Ratlar 3 gruba ayrılarak çalışıldı. Grup 1'e (n:6) sevofluran, %3 (v/v) hava/O₂ karışımı ile uygulandı. Ve grup 2'e (n:6) sevofluran + EGCg uygulandı. Kontrol grubu için ise yine 6 tane rat seçildi. Malondialdehid (MDA), nitrik oksit (NOx), süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px) and katalaz (CAT) kardiyak dokuda çalışıldı.

Bulgular: Grup 1'de CAT ve NO düzeyleri değişmezken, MDA, SOD ve GSH-PX düzeyleri yükselmiş olarak bulundu. EGCg'nin eklenmesi MDA, SOD ve GSH-Px aktivitelerini düşürürken, NOx düzeyleri artmış olarak bulundu.

Sonuç: Lipid peroksidasyonu ve antioksidan enzim düzeyleri sevofluran uygulamasını takiben fazla miktarda artmıştır. Ancak, iv EGCg uygulaması kardiyak dokuyu önemli şekilde korumaktadır. ©2007, Fırat Üniversitesi, Tıp Fakültesi

Anahtar kelimeler: EGCg, sevofluran, oksidan-antioksidan sistem

Part of the 'flurane' family, sevoflurane, is an inhalation anesthetic and is used for general anesthesia (1). Sevoflurane (SEV) has been reported to generate oxygen-derived free radicals, which can be one of the major causes for the impairment of nitric oxide (NOx)-induced relaxation of vascular smooth muscle (2). It has also been reported that SEV reduced endothelium-dependent vasorelaxation by generating superoxide anion (3). Recently, several studies have shown that ROS exist in sevoflurane induced cardioprotection (4,5). It is now commonly recognized that reactive oxygen species (ROS) are involved in a variety of physiological and pathological processes, including cellular signal transduction, cell proliferation and differentiation, apoptosis as well as ischemiareperfusion injury, inflammation and many degenerative diseases (6-9). Especially, ROS mediated lipid

peroxidation and DNA damage is associated with a variety of chronic health problems, such as cancer, ageing and atherosclerosis (10-12).

Tea is a rich source of polyphenolic compounds, particularly flavonoids. Flavonoids are phenols that are widely distributed in plants; more than 4000 have been identified (13). The main flavonoids present in green tea include catechins; among them, epigallocatechin 3-gallate (EGCg) has the highest antioxidant capacity. In addition, EGCg has many biological functions, including antioxidant activity (14), antimutagenic (15) and anticarcinogenic effects (16), and inhibitory action on tumour invasion and angiogenesis (17). Green tea is one of the most popular beverages in the world and has biologically important polyphenols. EGCg is the most active major polyphenol of green tea and primarily responsible for the green

tea effect. It has been proved EGCg shows a potent antioxidant property (18,19). EGCg possesses two triphenolic groups in its structure, which have been reported to be important for its potent antioxidant activity (20).

The aim of the study is to investigate the effects of sevoflurane on oxidants and antioxidant status of rat heart tissue. In this paper, we also have performed in vitro experiments to investigate the protective effects of EGCg against sevoflurane anesthetic exposure by evaluating levels of malondialdehyde (MDA), nitric oxide (NOx), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in cardiac tissue.

MATERIALS AND METHODS

Animals, diets, experimental design and data collection

A total of 18 Wistar-albino rats (approximately 200 g BW) obtained from Veterinary Control and Research Institute of Elazığ, Turkey were used in the study. Rats were housed in cages at room temperature and were submitted to light/dark cycle of 12 hours. Wistar-Albino rats were randomly assigned to one of three groups: Group 1 (n:6): sevoflurane; Group 2 (n:6): sevoflurane plus EGCg; Group 3 (n:6): control. Placebo (physiological saline, 0.9%) was given to animals in Group 1 and Group 3 by gavage and EGCg (Teavigo; 40 mg/kg/d) was given to animals in group 2 by gavage for ten days. After EGCg supplementation for 10 days, the group 1 and Group 2 rats were exposed to an anesthetic gas mixture. Sevoflurane (3%) (v/v) was given to animals in the vaporizer in air oxygen mixture 50% oxygen and 50% N₂O mixture (4L/minute). The gas mixture was administered for two hours in a continuous flow chamber, breathing spontaneously without any surgical maneuver. The anesthetic gases were administered via Dräger anesthetic machine (Draeger Cato-Edition, Lübeck, Germany). This Dräger anesthetic machine made by glass and its size was 40x40x70. During the study, these animals were fed ad libitum with a food including the ingredients shown in Table 1. The gas mixture was administered for two hours. Then Animals were killed by cervical dislocation, blood and tissue samples were taken into ice bath until homogenisation.

Tissue homogenisation and determination of tissue NOx, MDA, GSH-Px, CAT and SOD level

Heart was quickly removed, the blood being washed out with ice-cold 0.9% saline solution. For the determination, 1 gr tissues were homogenized in 9 mL Tris/HCl (0.02mM and pH 7.4), using an all-glass homogenizer. For determination of NO and MDA, homogenate was used. After centrifugation at 800×g for 20 min, the resulting supernatant fraction was used for determination of SOD, GSH-Px and CAT levels. The protein concentration of the homogenat and supernatant were determined by the method described by Lowry et al. (21). Plasma NOx levels were measured in triplicate after conversion of nitrate to nitrite by nitrate reductase, and nitrite was measured by using the Griess reaction, as described previously (22). The results of tissue were expressed as nmol/g wet tissue and the results of serum were expressed as nmol/ml. MDA, which is the end product of lipid peroxidation, was measured spectrophotometrically as described by Ohkawa et al (23).

A Part of the homogenate was extracted in ethanol/ chloroform mixture (5/3, v/v) to discard the lipid fraction, which caused interferences in the activity measurements of glutathione peroxidase. After centrifugation at 10.000 × g for 60 min, the upper clear layer was removed and used for the analyses. Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Durak et al. (24). GSH-Px activity levels were measured using the method of Paglia and Valentine (25) in which GSH-Px activity was coupled with the oxidation of NADPH by glutathione reductase. The oxidation of NADPH was followed spectrophotometrically at 340 nm. The tissue catalase (CAT) activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (26).

Statistical Analyses

All values were presented as mean ± standard deviation (SD). Statistical evaluation of each value was performed using one-way analysis of variance for multiple comparisons. Two-way ANOVA was performed to compare differences in groups. Values were considered statistically significant at P <0.05.

RESULTS

Tissue MDA, NOx, GSH-Px, CAT and SOD levels are shown in Table 2. Sevoflurane exposure increased MDA, SOD and GSH-Px activities (p<0.05, p<0.0001, p<0.001, respectively) while non-significant changes occurred in CAT and NOx activities when sevoflurane was exposed compared with control group. Supplementation of EGCg (group 2) decreased the MDA, SOD and GSH-Px activities whereas, the NOx activities increase with EGCg supplementation compared with group 1. There were no significant differences among groups in CAT activities.

Table 1. Ingredients and chemical analyses of the starter and grower diets fed to quails, g/100 g

Ingredients	
Corn	41.45
Wheat	10.50
Soybean meal	25.0
Fishmeal	10.0
Wheat bran	10.0
Limestone powder	0.55
Sodium chloride	0.50
¹ Vitamin premix	1.00
*Mineral premix	1.00

¹Vitamin premix provides the following per kilogram: all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-α-tocopherol acetate, 1.25 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; d-biotin, 0.1 mg.

*Mineral premix provides the following per kilogram: manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

Table 2. Levels of MDA, NO, SOD, GSH-Px and CAT activities in hearth tissue

	Group 1 Sevoflurane	Group 2 Sevoflurane+EGCg	Group 3 Control
MDA (nmol/grwettissue)	33.33±9.3 ^a	19.71±9.0	28.62±13.4
NO (µmol/grwettissue)	626.50±96.2 ^b	832.16±56.6 ^c	651.0±66.9
SOD (U/mgrprotein)	0.36±1.3 ^{b,d}	0.07±0.02 ^e	0.19±0.1
GPX (U/gprt)	16.29±1.0 ^c	13.85±2.6 ^e	10.95±2.9
CAT (k/gprt)	5.31±2.32 ^a	7.82±1.3	7.06±1.2

^aP<0.05, compared with Sevoflurane+EGCg

^bP<0.0001, compared with Sevoflurane+EGCg

^cP<0.001, compared with control

^dP<0.005, compared with control

^eP<0.05, compared with control

DISCUSSION

Free radicals such as superoxide radical or hydroxyl radical are constantly produced as a normal consequence of aerobic metabolism (27). Oxidative stress results from an imbalance between radical generating and radical-scavenging systems leading to cell membrane impairment or DNA damage (27). MDA is a reflection of lipid peroxidation, whereas SOD and GSH-Px are important antioxidant defenses. These enzymes are involved in the clearance of superoxide and hydrogen peroxide (H₂O₂) to maintain the structure and function of biological membranes (27). SOD dismutates superoxide H₂O₂ and this compound is catabolized by catalase and GSH-Px. In higher organisms, GSH-Px appears to have largely supplanted the need for catalase membranes (27). Thus, our findings support the existence of oxidative stress from mechanically ventilated animals during exposure to sevoflurane. Moreover, anesthesia conducted with EGCg reduced oxidative stress and enhanced antioxidant defense mechanisms expressed by larger concentrations of free radical scavengers.

Oxidative stress leads to the accumulation of lipid peroxidation products MDA in the heart, and causes impaired cell function, while antioxidant enzyme SOD and GSH-Px play great roles in cellular defense against oxidative stress. In this study, to confirm the presence of increased oxidative stress in cardiac tissue from mechanically ventilated animals during exposure to sevoflurane, we quantified myocardial levels of MDA, NO content, and SOD, GSH-Px and CAT activities. The results showed that the level of MDA, GSH-Px and SOD in cardiac tissue increased, while the activities of NO and CAT was not changed vs the sham-operated control, indicating a significant oxidative stress. The treatment with EGCg almost completely prevented the Sevoflurane effects in SOD and GSH-Px levels, and MDA formation both in cardiac tissue. These results suggest that the protective effects of EGCg on cardiac tissue were correlated. EGCg can generate H₂O₂ (28,29), and H₂O₂ can lead to eNOS activation and vasorelaxation in aortic rings (27). In a recent study (30) demonstrates that endothelium-dependent vasorelaxation induced by the tea-derived catechin EGCg occurs in response

to a potent, dose-dependent activation of eNOS in endothelial cells. The resulting increase in eNOS activity is observed within a few minutes, suggesting posttranslational regulation of eNOS as an underlying mechanism. In agree with this study, we also found that level of NO in cardiac tissue increased with EGCg supplementation.

Impairment of non-enzymatic antioxidant scavenging activity is related to elevation of toxic metabolites such as superoxide free radicals (O₂⁻), hydrogen peroxide (H₂O₂), or hydroxyl radicals (•OH). Elevation of O₂⁻, which is the substrate of SOD might be the reason of the higher enzyme activity. Also H₂O₂ which is produced by SOD might be the cause of CAT and GSH-Px production and higher activity of this enzyme with the same mechanism. In this study, we observed an elevation of SOD and GSH-Px levels in group I. Elevation of SOD and GSH-Px activities due to increased production of free radicals such as O₂⁻ and •OH were also observed. However these compensatory mechanisms were not able to prevent cellular lipid peroxidation. Therefore, these findings might be as a result of more production of radicals due to sevoflurane administration. Another finding supporting this hypothesis is MDA levels which have the highest values in group 1 Enzymatic findings may be important signs of effects of administration of sevoflurane and EGCg at the cellular level.

The volatile anaesthetic sevoflurane protects the heart against ischaemia-induced adenosine triphosphate (ATP) depletion, Ca²⁺ overload and oxidative stress through activation of protein kinase C (PKC), opening of mitochondrial K⁺ ATP channels (mitoK⁺ATP) and the production of ROS (4, 31-33). But Sevoflurane (34) have been proposed to cause formation of ROS directly in cardiac tissues. It may be possible that an anesthetic can cause free radical release from itself or the tissue with which it interacts.

In conclusion, the amount of lipid peroxidation and antioxidant enzyme activities were more increased in following sevoflurane administration in the 3% concentration. The administration of intravenous EGCg (40 mg/kg/d) significantly protected cardiac tissue. These conclusions were supported by the improved reduced levels of MDA.

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