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EGCg Supplementation Improves Oxidant and Antioxidant Status in Kidney of Rats Exposed to Sevoflurane

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ABSTRACT

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

Materials and Methods: Tree groups of animals were studied. Sevoflurane 3% (v/v) in air/O_2 were administered to animals in group 1 (n = 7) and sevoflurane plus EGCg was administered in group 2 (n = 7). Seven animals were allocated to control group (group 3). Malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in kidney tissue were studied. Histopathological changes were also assessed.

Results: In group I, MDA, NO, SOD and GSH-Px activities were increased while decreased CAT activities occurred when sevoflurane was exposured compared with controls. Supplementation of EGCg (group 2) decreased the MDA ($P<0.05$), SOD ($P<0.005$) and GSH-Px activities whereas, the NO ($P<0.005$) and CAT ($P<0.005$) activities increase with EGCg supplementation compared with group 1. In histopathology, sevoflurane exposure group had severe degeneration with moderate cortical necrosis in kidney tissue. Administration EGCg with sevoflurane exposure decreased degeneration.

Conclusion: The amount of lipid peroxidation and antioxidant enzyme levels were more increased in following sevoflurane administration. The administration of EGCg significantly protected kidney tissue.

Key word: *EGCg, Sevoflurane, oxidant-antioxidant system.*

ÖZET

EGCg İlavesi Sevofluran Uygulanan Ratların Böbreklerinde Oksidan ve Antioksidan Durumunu Düzenler

Amaç: Ratlarda böbrek dokusu üzerinde oksidan ve antioksidan durumu değerlendirerek sevofluran anestezisi ve epigallocatechin 3- gallate (EGCg)'ın muhtemel etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Ratlar 3 gruba ayrılarak çalışıldı. Grup 1 (n:7): Sevofluran, %3 (v/v) hava/O_2 karışımı ile uygulandı. Grup 2 (n:7): Sevofluran + EGCg uygulandı. Kontrol grubu için ise yine 7 tane rat seçildi. Malondialdehid (MDA), nitrik oksit (NO), süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve katalaz (CAT) böbrek dokusunda çalışıldı. Ayrıca histopatolojik değişiklikler değerlendirildi.

Bulgular: Grup 1'de MDA, NO, SOD ve GSH-Px aktiviteleri artmışken, CAT aktivitesi sevofluran uygulananlarda azalmıştır. EGCg'nin uygulaması (grup 2) MDA, SOD ve GSH-Px aktivitelerini azaltırken, NO ve CAT aktiviteleri grup 1 ile karşılaştırıldığında artmış olarak bulunmuştur. Histopatolojide sevofluran uygulanan grupta böbrek dokusunda orta derecede kortikal nekroz ve şiddetli dejenerasyonla karakterize değişimlerin oluştuğu saptandı. Sevofluran ile birlikte EGCg uygulamasının ise dejenerasyonu azalttığı bulunmuştur.

Sonuç: Lipid peroksidasyonu ve antioksidan enzim düzeyleri sevofluran uygulamasını takiben fazla miktarda artmıştır. Ancak, EGCg uygulaması böbrek dokusunu önemli ölçüde korumaktadır.

Anahtar kelimeler: *EGCg, Sevofluran, oksidan-antioksidan sistem*

There are many reports of the renal effects of sevoflurane. Importantly, both compound A and inorganic fluoride (F-) are produced during sevoflurane anesthesia, and both might be nephrotoxic (1-3). Compound A is a degradation product of sevoflurane produced by the reaction of sevoflurane with strong bases in the carbon dioxide absorbents (4), and its role in nephrotoxicity has been reported. Histologic evidence of renal injury was observed in rats exposed to compound A (5,6). In human studies, only transient renal damage has been reported with sevoflurane anesthesia (7,8). Compound A is not a product of hepatic metabolism, whereas F- is produced in the liver. A degree of the renal tubular damage correlated well with the total amount of F- exposed to the kidney; that is, the greater the amount of F- , the greater the renal tubular damage (3). However, there are no reports of clinically significant renal failure after sevoflurane anesthesia.

Green tea has highly reputed chemotherapeutic effects and is one of the most widely investigated herbs. Since it has been imbibed in China, Korea and Japan for thousands of years, its long-term safety is well established. Its wide safety margin makes it one of the safest herbal medicines available

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(9). The main constituents of green tea extract are catechins: (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (−)-gallocatechin gallate (GCG) and (−)-epigallocatechin gallate (EGCg). The most important property of catechins is their antioxidative ability to scavenge free radicals from damaging biomolecules, chelate catalytic metal ions from free radical formation and quench singlet oxygen from activating organic molecules to form peroxides and free radicals (10-12). Such properties prevent DNA damages by reactive oxygen species. Catechins are therefore both anti-mutagenic and anticarcinogenic (9). The main flavonoids present in green tea include catechins; among them, EGCg has the highest antioxidant capacity. The aim of the study is to investigate the effects of sevoflurane on oxidants and antioxidant status in the kidney 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 (NO), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in kidney tissue and by evaluating histopathology.

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 Elazig, Turkey were used in the study. Rats were housed in cages at room temperature and were submitted to light/dark cycle of 12 hours. The rats were randomly assigned to one of tree groups: Group 1 (*n*:7): sevoflurane; Group 2 (*n*:7): sevoflurane plus EGCg; Group 3 (*n*:7): 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 suplementation 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 (50/50) at 4L/min for 2 h. 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. The anesthetic gas mixture was exposed to athmosphere air by pipe. 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 NO, MDA, GSH-Px, CAT and SOD level

Kidney was quickly removed, the blood being washed out with ice-cold 0.9% saline solution. For the determination of oxidant and antioxidants, 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. (13). Plasma NO levels were measured after conversion of nitrate to nitrite by nitrate reductase, and nitrite was measured by using the Griess reaction, as described previously (14). The results of tissue were expressed as nmol/g wet tissue MDA, which is the end product of lipid peroxidation, was measured spectrophotometrically as described by Ohkawa et al (15). 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.* (16). GSH-Px activity levels were measured using the method of Paglia and Valentine (17) 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 CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (18).

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

[1]Vitamin premix provides the following per kilogram: all-trans-retynyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-α-tocoperol acetate, 1.25 mg; menadione (menadione sodium bisulfate, 1.1 mg; riboflavin, 4, 4 mg; thiamine (thiamine mononitrate)$_1$, 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 potasium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg

Histopathological evaluation

Kidney tissue was fixed in 10% buffered formaldehyde for histopathology evaluation. The formaldehyde-buffered kidney tissues were embedded in paraffin and at least four cross-sections were taken from kidney in 4–5 ml thickness and stained with hematoxyline–eosin (H and E). Histopathological examination was performed by a pathologist blinded to the study groups.

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, NO, GSH-Px, CAT and SOD levels are shown in Table 2. Sevoflurane exposure increased MDA, NO, SOD and GSH-Px activities while decreased CAT activities occurred but these were not statistically significant compared with controls.

Supplementation of EGCg (group 2) decreased the MDA (P<0.05), SOD (P<0.005) and GSH-Px activities whereas, the NO (P<0.005) and CAT (P<0.005) activities increase with EGCg supplementation compared with group 1.

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

	Group 1 Sevoflurane (n:7)	Group 2 Sevoflurane+EGCg (n:7)	Group 3 Control (n:7)
MDA (nmol/grwettissue)	33.36±10.85[a]	19.84±8.10	24.40±9.91
NO (µmol/grwettissue)	688.05±47.47[b]	867.51±115.85[c]	606.97±111.51
SOD (U/mgrprotein)	0.24±0.6[b]	0.10±0.06	0.19±0.11
GSH-Px (U/grprotein)	20.07±13.95	15.61±6.22	12.33±3.32
CAT (k/grprotein)	23.44±4.37[b]	40.64±12.15[d]	27.12±9.37

[a] P<0.05, compared with group 2
[b] P<0.005, compared with group 2
[c] P<0.0001, compared with group 3
[d] P<0.05, compared with group 3

Histopathology

In sevoflurane exposure group, there was severe degeneration with moderate cortical necrosis in kidney tissue. In kidney tissue, there was degeneration and desquamation with intertubular hemoragie in proximal tubulus epitel. Despite of degeneration, there were picnotic nucleuses in tubulus cells. Hyalin cylinders were shown in the lumen of tubules. Also, in the medulla of the kidney, severe congestion with intertubular hemoragie was shown (Figure 1).

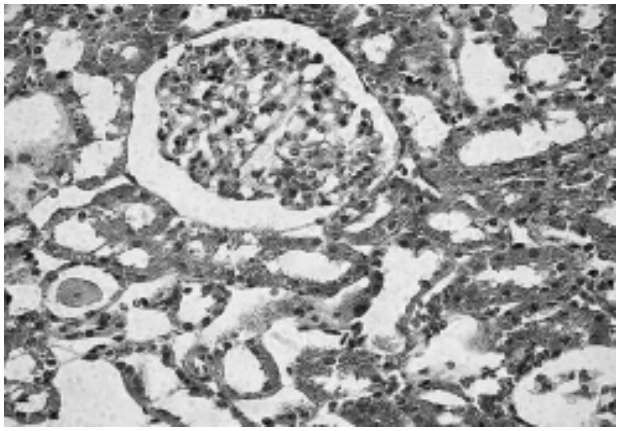

Figure 1. After sevoflurane exposure, severe degenerative and necrotic changes in proximal tubulus epitel in the kidney **(H&E, × 200).**

Administration EGCg with sevoflurane exposure decreased the degeneration and hyalin cylinder formation didn't show in the tubuler lumen (Figure 2).

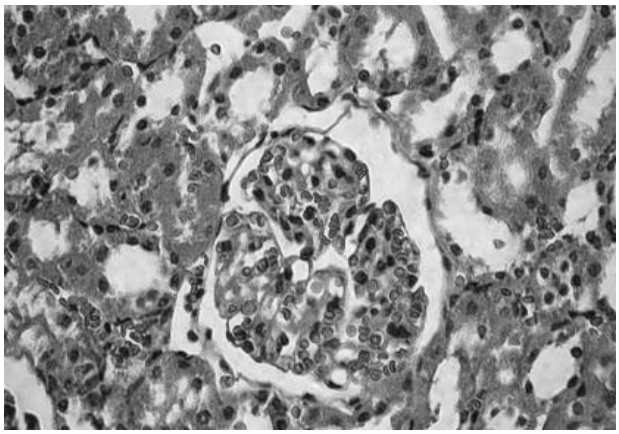

Figure 2. After administration EGCg with sevoflurane, mild degenerative changes **(H&E, × 200).**

DISCUSSION

In the present study, we also evaluated the effect of ECGg supplementation on biomarkers related to the antioxidant defence system and antioxidant enzymes in animals exposed to sevoflurane. It is well known free radical species (FRS) are constantly produced as a normal consequence of aerobic metabolism. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, leading to cell membrane impairment or DNA damage. MDA is a reflection of lipid peroxidation. Protein carbonyl content is evaluated as an index of protein oxidation. Protein SH groups are important antioxidant defenses (19). Increased lipid peroxidation and decreased antioxidant protection may lead to cytotoxicity, allergy, mutagenicity, and carcinogenicity (20). 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 H_2O_2 to maintain the structure and function of biological membranes (19). SOD dismutases superoxide H_2O_2 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 (19). Thus, our findings support the existence of a local and systemic oxidative stress from spontaneous ventilated animals during exposure to sevoflurane. Moreover, anesthesia conducted with propofol reduced oxidative stress and enhanced antioxidant defense mechanisms expressed by larger concentrations of free radical scavengers. Sivaci and et al showed that antioxidant activity is affected after sevoflurane anesthesia whereas antioxidant activity is reduced significantly after desflurane anesthesia (21).

In a recent study (22) 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 kidney tissue incresed with EGCg supplementation. It has been showed that catechins chelating properties could also have a fundamental role in preventing peroxidation (23).

Dikmen et al (24) observed an elevation of SOD, GSH-Px and CAT levels in anasthesic groups they reported that elevation of SOD, CAT and GSH-Px activities due to increased production of free radicals. In the present study, our results show that animals exposed to sevoflurane had increased MDA,

NO, SOD and GSH-Px concentrations in kidney tissue, but the increased level of GSH-Px was not statically significant. Conversely, EGCg supplementation decreased MDA, SOD and GSH-Px levels in animals exposed to sevoflurane. We could not find any previous report on the effects of EGCg supplementation on kidney tissue levels of MDA, NO and antioxidant enzymes in rats exposed to anasthesic agents and to compare with our results. Against all the protective enzymatic mechanisms, cellular damage that supports the elevation of lipid peroxidation was observed in group 1. Histopathological findings in group 1, there was severe degeneration with moderate cortical necrosis in kidney tissue. Administration of sevoflurane plus EGCg in rats, decreased degeneration in histopatological findings.

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 EGCg (40 mg/kg/d) significantly protected kidney tissue. These conclusions were supported by the improved reduced levels of MDA.

REFERENCES

1. Gonsowski CT, Laster MJ, Eger EI, Ferrell LD, Kerschmann RL. Toxicity of compound A in rats: effect of increasing duration of administration. Anesthesiology 1994; 80: 556-565.
2. Higuchi H, Arimura S, Sumikura H, Satoh T, Kanno M. Urine concentrating ability after prolonged sevoflurane anaesthesia. Br J Anaesth 1994; 73: 239-240.
3. Bito H, Ikeda K. Plasma inorganic fluoride and intracircuit degradation product concentrations in long-duration, low-flow sevoflurane anesthesia. Anesth Analg 1994; 79: 946-951.
4. Morio M, Fujii K, Satoh N, et al. Reaction of sevoflurane and its degradation products with soda lime. Anesthesiology 1992; 77: 1155-64.
5. Kandel L, Laster MJ, Eger EI, Kerschmann RL, Martin J. Nephrotoxicity in rats undergoing a one-hour exposure to compound A. Anesth Analg 1995; 81: 559-63.
6. Martin JL, Laster MJ, Kandel L, et al. Metabolism of compound A by renal cysteine-S-conjugate beta-lyase is not the mechanism of compound A-induced renal injury in the rat. Anesth Analg 1996; 82: 770-774
7. Eger EI, Gong D, Koblin DD, et al. Dose-related biochemical markers of renal injury after sevoflurane versus desflurane anesthesia in volunteers. Anesth Analg 1997; 85: 1154-1163.
8. Eger EI, Koblin DD, Bowland T, et al. Nephrotoxicity of sevoflurane versus desflurane anesthesia in volunteers. Anesth Analg 1997; 84: 160-168.
9. Mitscher LA, Jung M, Shankel D, et al. Chemoprotection: a review of the potential therapeutic antioxidant properties of green tea (Camellia sinensis) and certain of its constituents. Med. Res. Rev. 1997; 17:327-365
10. Rice-Evans C. Plant polyphenols: free radical scavengers or chain-breaking antioxidants? Biochem Soc Symp. 1995; 61: 103-116.
11. Kuo SM, Leavitt PS, Lin CP. Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. Biol. Trace Element Res. 1998; 62: 135-53.
12. Yoshino M, Murakami K. Interaction of iron with polyphenolic compounds: application to antioxidant characterization. Anal. Biochem. 1998; 257: 40-44.
13. Lowry O, Rosenbraugh N, Farr L,Randall R. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 182, 265–275
14. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990; 36: 1440-1443.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.Analytical Biochemistry. 1979; 95: 351-358.
16. Durak I, Yurtarslani Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clin Chim Acta; 1993; 214: 103-104.
17. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. J Lab & Clin Med 1967; 70: 158-169.
18. Aebi H. Catalase In: Bergmeyer U, ed. Methods of enzymatic analysis. New York and London: Academic Press; 1974; 673-677.
19. McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000; 108: 652–659.
20. Matés JM, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem 1999; 32: 595–603.
21. Sivaci R, Kahraman A, Serteser M, Sahin DA, Dilek ON. Cytotoxic effects of volatile anesthetics with free radicals undergoing laparoscopic surgery. Clin Biochem. 2006; 39: 293-298.
22. Lorenz M, Wessler S, Follmann E, et al. Constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase- cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J Biol Chem 2004; 13: 6190-6195.
23. Frei B., Higdon J.V. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies, J. Nutr. 2003;133: 3275–3284
24. Dikmen B, Unal Y, Pampal HK. Effects of repeated desflurane and sevoflurane anesthesia on enzymatic free radical scavanger system. Molecular and Cellular Biochemistry, 2007; 294: 31-36.

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