Effect of Sevoflurane on Enzymatic Antioxidant Defense System in Guinea Pig Liver

SEVOFLURANIN KOBAY KARACİĞERİNDE ENZİMATİK ANTİOKSİDAN SAVUNMA SİSTEMİNE ETKİSİ

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Summary

Background and aim: Hepatic dysfunction after sevoflurane anesthesia has been reported previously. In this study, possible effects of sevoflurane on the enzymatic antioxidant defense system was investigated in guinea pig liver.

Materials and Methods: Three groups of seven animals were studied: I-control, II-sevoflurane, and Ill-oxygen. Animals in oxygen group were treated with 100% oxygen and animals in sevoflurane group with sevoflurane (1.5% v/v) in oxygen (100%, 2 L/min) for 30 minutes for consecutive three days. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) enzyme activities and malondialdehyde (MDA) levels were measured in the liver tissues of animals.

[**Results:** None of the enzymes' activities (SOD, CAT, GSH-Px) were found to be significantly changed, but the MDA level was found to be significantly ($p<0.0005$) increased in the sevoflurane group compared to the control group.

<ZTonclusion: Sevoflurane induced an acceleration in lipid peroxidation without a failure in the enzymatic antioxidant defense system.

^ **Cey Words:** Sevoflurane, Liver, Antioxidant enzymes

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Özet

Amaç: Sevofluran anestezisini takiben ortaya çıkan hepatik disfonksiyon, bazı araştırmacılar tarafından evvelce bildirilmişti. Bu çalışmada, sevofluranın kobay karaciğerinin enzimatik antioksidan savunma sistemi üzerindeki muhtemel etkileri araştırıldı.

Materyal ve Metod: Her grupta yedişer hayvan bulunan 3 grup oluşturuldu: I- kontrol, II- sevofluran, III- oksijen. Oksijen grubunda bulunan hayvanlar %100 oksijene, sevofluran grubunda bulunanlar ise oksijen içinde (%100, 2 L/dk) sevoflurana (%1,5 v/v) ardarda 3 gün, 30 dk süre ile maruz bırakıldı. Hayvanların karaciğer dokularında süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-Px) enzim aktiviteleri ve malondialdehid (MDA) seviyeleri tayin edildi.

Bulgular: Kontrol grubu ile karşılaştırıldığında sevofluran grubunda, her üç enzimin (SOD, CAT, GSH-Px) aktivitesinin de değişmediği, fakat MDA seviyesinin anlamlı bir şekilde arttığı ($p<0,0005$) gözlendi.

Sonuç: Sevofluran, enzimatik antioksidan savunma sisteminde bir yetersizlik ortaya çıkarmaksızın lipid peroksidasyonunda artışa neden oldu.

Anahtar Kelimeler: Sevofluran, Karaciğer, Antioksidan enzimler

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Hepatic dysfunction after sevoflurane anesthesia has been reported previously (1-5). The hepatotoxic effect of sevoflurane however was not suggested to be through the same mechanism as in halothane hepatitis. It is known that halothane is oxidatively metabolized to trifluoroacetylchloride (CF_3COCl) by cytochrome P450 2E1, which may then bind covalently to polypeptides (6-8) and produce trifluoroacetylated proteins (7,9-11) in the liver. An immune response directed against those

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Table 1. Mean±SD values of SOD (U/mj*l),* CAT (IU/mg), GSH-Px (IU/mg) enzyme activities and MDA (nmol/mg) levels in liver tissues from guinea pigs

Groups	SOD	CAT	GSH-Px	MDA
I(n=7)	18.9±1.2	0.22±0.02	0.05±0.01	0.31 ±0.04
II (n=7)	20.1±4.1	0.20±0.03	0.06±0.01	0.42±0.05
III (n=7)	19.7±4.3	0.14±0.04	0.06±0.01	0.34±0.06
ANOVA	F=0.213 p>0.75	F=0.404 p<0.75	F=2.345 p<0.25	F=8.852 p<0.005
		student's t-test		
I-II	n.s.	n.s.	n.s.	0.0005
I-III	n.s.	n.s.	n.s.	n.s.
II-III	n.s.	n.s.	n.s.	0.01

I-Control, II-Sevoflurane, Ill-Oxygen
n.s.: non-significant (p>0.05)

modified proteins (CF_3CO-proteins) can cause hepatitis. Unlike halothane, enflurane and isoflurane; sevoflurane does not undergo metabolism to reactive acyl halide intermediates, which covalently bind to liver proteins. Instead, it undergoes biotransformation to inorganic fluoride and organic fluoride metabolite hexafluoroisopropanol (HFIP) (12,13). HFIP does undergo conjugation with glucuronic acid, which is excreted in urine (12,13). There is no evidence for HFIP toxicity at clinically produced concentrations. In a previous study Yoshida and Okabe reported that, oxygen free radicals were generated by sevoflurane treatment (14), which suggests that this effect of sevoflurane might also contribute to its hepatotoxic effect.

In this study, we investigated the possible effects of sevoflurane on the enzymatic antioxidant defense system in guinea pig liver.

Materials and Methods

Three groups of seven animals (two months old, approx. 450 g weight) were studied: I-control, II-sevoflurane, and Ill-oxygen. Animals in the oxygen group were treated with 100% oxygen and animals in the sevoflurane group with sevoflurane (1.5% v/v) in oxygen (100%, 2 L/min) for 30 minutes for consecutive three days. The animals were fed a laboratory diet during the study. The gas mixture was delivered by using facemasks. At the end of the experiments, two hours after the last sevoflurane treatment, the animals were killed and their livers were removed and placed in an ice bath until homogenization for about one hour.

Biochemical analysis: Livers were first washed with deionized water to remove blood and then homogenized in a homogenizer (B.Braun Melsungen model) at 1000 U for about three minutes. During the tissue preparation procedure, the temperature was +4°C. After centrifugation at 10,000 g for about 60 min, the upper layer was taken for analysis. In this fraction SOD, CAT and GSH-Px activities were measured as described, respectively (15-17). One unit of SOD activity was defined as the amount of protein causing 50% inhibition in nitroblue tetrazolium salt (NBT) reduction rate. The CAT and GSH-Px activities were given in IU/mg protein and SOD activity in U/mg protein. The MDA level was determined by using thiobarbituric acid reaction (18) and the amount of protein by the Lowry method (19).

In the statistical analysis, ANOVA and student's t-test were used.

Results

Results are given in Table 1. As seen from the table, SOD, CAT and GSH-Px enzyme activities were found to be unchanged, but the MDA level was found to be significantly (p<0.0005) increased in the sevoflurane group compared to the control group.

Discussion

Sevoflurane has been reported to induce some hepatotoxic effects in several case reports (1-5). However, no satisfactory explanation was given for the molecular mechanism. Yoshida and Okabe reported that sevoflurane could induce oxygen free radical generation (14), which suggests that it may have hepatotoxic effects. For testing the latter suggestion, we used a multiple exposure animal model and measured antioxidant enzyme activities and MDA levels (as a peroxidation index) in the liver tissues of guinea pigs. None of the antioxidant enzyme activities were found to be changed however, the MDA level was increased significantly after sevoflurane treatment. As far as we know, no previous study has reported the induction of lipid peroxidation after sevoflurane treatment in liver tissue. This increase in MDA concentration indicates an acceleration in lipid peroxidation, which is not caused by a failure of the enzymatic antioxidant defense (unchanged enzyme activities). An increase in lipid peroxidation without enzymatic antioxidant defense failure may have two possible causes: 1-Sevoflurane may cause non-enzymatic antioxidant depletion and/or 2-Some radicalic compounds may be produced as a by-product while sevoflurane is metabolized. To elucidate the exact molecular mechanism, this subject needs further investigation.

In conclusion, sevoflurane treatment induces an acceleration in lipid peroxidation in liver without a failure in the enzymatic antioxidant defense system.

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