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The effect of sevoflurane and desflurane on lung tissue damage after distant organ ischemia and reperfusion in diabetic rats

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Abstract
Objectives: Desflurane and sevoflurane are commonly used as inhaler agents in anesthesia. Although the protector effects of volatile anesthetics on the brain, heart and liver are well known, their effects on distant organ induced injuries in the lungs is not clearly understood. We examined the lungs, about the iscemia-reperfusion (I/R) distant organ damage for sevoflurane and desflurane.
Methods: Diabetic animals were randomly divided into five groups, which had six animals. The groups were like this; first group was control group without diabetes. Second group was diabetic control group (DC), Third group was diabetic I/R (D-I/R), Fourth group was diabetic I/R group with desflurane(D-I/R-D), and the fifth group was diabetic I/R group with sevoflurane (D-I/R-S)
Results: Neutrophile infiltration/aggregation rate was fairly high in the DIR group when compared to other four groups DC, C, DIRS and DIRD. ($p<0.0001$, $p<0.0001$, $p=0.00+95$, $p=0.005$, respectively), Alveolar wall thickening in lung tissue was found significantly higher in the DIR group compared to the C and DC groups ($p<0.0001$, $p<0.0001$, respectively). The lung tissue injury score was found significantly higher in the DIR group compared to the C, DC, DIRD and DIRS groups ($p<0.0001$, $p<0.0001$, $p=0.004$, $p=0.004$, respectively),
Conclusion: Sevoflorane and desflorane have positive effects on MDA, SOD and LTIS parameters in I/R damage on the distant organ lungs.

Keywords: ischemi reperfusion, desflurane, sevoflurane, lung

1. Introduction
Ischemia reperfusion injury is the damage given to tissues by reactive oxygen radicals that form together with reoxygenization after anoxia in organs [1]. This damage may be in the directly affected organ or may be in distant organs as the result of reactive oxygen radicals released to blood such as superoxide, hydrogen peroxide and hydroxy radicals [2, 3]. Damage may occur in many organs including lungs, liver, kidney and pancreas after ischemia reperfusion injury [4]. The most commonly affected organ is the lung. Diabetic backround aggravates ischemia reperfusion process with lipid peroxidation and causes serious injury in distant organs with free oxygen radicals released to the medium [5, 6].
Desflurane and sevoflurane are commonly used as inhaler agents in anesthesia. The most important feature of desflurane is the rapid start and end of its effect [7]. Sevoflurane is widely used especially in induction anesthesia and maintenance of anesthesia due to rapid uptake and rapid elimination.
Although protective effects of volatile anesthetics on the liver, brain and heart have been demonstrated, their effect on the distant organ lungs have not been clearly understood [7, 8]. Fernanda *et al*. reported that sevoflurane decreases ischemia reperfursion injury in the liver with its anti-inflammatory features as the other volatile general anesthetics [9].
In this paper, the affect of sevoflurane and desflurane on ischemia and reperfusion damage on distant organ induced injuries in the lungs, were researced on.

2. Material and Methods
The protocol of experiment and animals
The research was done after ratification of Gazi University Experimental Animals Ethics Committee in Gazi University Experimental and Clinical Research Center (GUDAM). Accepted standards of the guide for care and use of laboratory animals were used for all procedures. For this research in the range of 200 and 250 g, 30 male Wistar albino rats were used which are raised under the same environmental provisions. The rats were exposed to cycles of 12h daylight and 12h darkness and were able to reach food until 2h before anesthesia given. Six rats without diabetes were control group and the other diabetic rats were divided into four groups randomly,having six rats. A diabetic kontrol group (DC), a diabetic I/R group (DIR), a diabetic I/R group with desflurane (DIRD), and a diabetic I/R group with sevoflurane (DIRS). Streptozotocin STZ (Sigma Chemicals, St. Louis, MO, USA) was used for treatment i and prepared just before the treatment by dissolving it in saline solution (0.9% NaCl). After three days applying STZ, the levels of blood glucoses were evaluated and the rats were classifiedas diabetic which glucose (FBG)

levels are over 250mg/dl. Before applying desflurane and sevoflurane the rats were observed four weeks allowing to have chronic diabetes, after the STZ injection [10].

I/R

Anesthetic gas vaporizers were calibrated and set a minimum alveolar concentration (MAC) of 1 and sevoflurane (%2) and desflurane. The anesthesia procedure was conducted with the rats in a transparent plastic box of 40X40X70 cm in size. The box which allowed for observation of the rats, was connected to a half-open anesthesia machine with static hoses. The anesthetic gases were released into the container in100% O2. The rats were divided into five groups all including six rats. Desflurane was applied with the 6% inspiratory concentration at a rate of 6 L.min-1 in 100% O2 for 2 hours, and sevoflurane was applied with the 2% inspiratory concentration at a rate of 6 L.min-1 in 100% O2 for 4 hours. Control group (C), Diabetic control group and DIR group had not any administration. After shaving the abdomen, all rats were positioned as supine on operating table. A median laparatomy was applied after cleaning the abdomen region with 1% polyvinylidene and covered with drape.The infrarenal segment of the aorta was clamped for 2h.after removing the clamp blood supplied for another two hours. After this reperfusion time, biochemical and histopathological evaluations of lung tissue spesimens were performed.Rats were decapitated at the end of the experiment. Histopathological assesment was done in Kırıkkale university Medical Faculty histology and embryology department. Paraffin blocks were formed by the specimens after routine fixation process. Tissue sections were mounted on slides for staining with hematoxylin and eosin

Histopathological assesment of the Lung

A researcher who has not an idea about the study, examined the all lung samples histopathologically by light microscopy. Ten random areas were evaluated using microscopy in (H&E)-stained sections. Neutrophil infiltration and alveolar thickness are measured in each spesimen for exposing the degree of lung injury area. Each parameter was scored as any (0point), Quite little (1 point), middle (2 points), or severe (3 points); The two scores were added and noted as last lung injury score. The biochemical evaluation was done in Gazi University Medical Faculty Medical Biochemistry Department. Malondialdehyde (MDA) levels in lung tissue are used for determining oxidative stress and lipid peroxidation. Superoxide Dismutase (SOD) activities were also measured [12].

Statistical Analyses

All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 for Windows and p <0.05.was considered significant. Each Categorical variables were analyzed by Kolmogorov-Smirnov test to compare all groups. Variations in MDA levels, SOD activities and histopathological parameters were evaluated by using the Kruskal-Wallis test. Bonferroni adjusted Mann-Whitney U test was when the Kruskal-Wallis test was significant to determine groups which difered from others. Results were expressed as mean± standard deviation (Mean ± SD).

3. Results

There were significant difference among the groups according to neutrophil infiltration/aggregation (p<0.0001). When compared to C, DC, DIRS and DIRD groups, ın DIR group neutrophile infiltration or aggregation was significantly higher (p<0.0001, p<0.0001, p=0.005, p=0.005, respectively). Table 1and (Figure 1-4). Alveolar Wall thickening was significantly higher in DIR group from C and DC groups (p<0.0001, p<0.0001) (Table 1), (Figure 1-4). Additionally alveolar Wall thickening was significantly higher in all groups except DC, when compared to control group (DIR p<0.0001, DIRD p=0.003, DIRS p=0.003). When the lung injury scores were compared it was significantly higher in DIR group from the other groups.(C p<0.0001, DC p<0.0001, DIRD p=0.004, DIRS p=0.004) (Table 1), (Figure 1-4).

Additionally the lung tissue injury score was significantly higher in the DIR,DIRD, and DIRS groups compared to C group (p<0.0001, p=0.002, p=0.002, respectively). When the groups were compared to each other fort he MDA levels, a significant difference was noticed (p<0.0001). MDA level was significantly higher in the DIR group compared to the C, DC, DIRD and DIRS groups (p<0.0001, p<0.0001, p<0.0001, p<0.0001, respectively), (Table 2).

When the SOD enzyme activity was compared among the groups, an important difference was observed (p<0.0001). The SOD enzyme activity was significantly higher in DIR group when compared to all other groups.(C, DC, DIRD and DIRS) (p<0.0001, p=0.007, p=0.003, p=0.002, respectively), (Table 2).

4. Discussion

Lung damage is known to occur because of the systemic inflammatory response which develops after ischemia reperfusion in the organs other than the lungs. Primary effects of oxygen radicals released to the medium on the lungs include vascular endothelial injury, increased permeability and ultimately pulmonary edema [2, 3]. Although many studies have used different parameters, lung damage has been evaluated especially with light microscopic findings, histopathological staining and biochemical parameters.

SODs protects cells against the damage caused by oxidative stress which occurs with ischemia reperfusion by acting as an antioxidant in the cell [12, 13]. Therefore, evaluation of SOD activity is considered as an important indicator. In our study, significant SOD enzyme activity found in DIR group compared with the other group could be considered as an indicator of lower damage in desflurane and sevoflurane groups. Similarly malondialdehyde is one of the final products of lipid peroxidation. It also known as a marker for peroxidation of the cell wall. The levels of MDA in plasma and tissue, are accepted to be a good marker for oxidative stress and the systemic response which occurs following ischemia and reperfusion [9, 13]. Increasing of lipid peroxidation causes the releasing of preteolytic lysosomal enzymes andd mitochondrial matrix enzymes to the plasma. This is one of the important causes of cellular damage [6].

In this study MDA levels were higher in DIR group than sevoflurane and desflurane groups.

In our study, we found significant difference in the histopathological examination between the groups in terms of lung tissue neutrophil infiltration/aggregation, alveolar wall thickness and lung tissue injury score (LTIS). Alveolar

wall thickness was significantly increased in DIR group, while neutrophil infiltration/aggregation and LTIS were lower in sevoflorane and desflorane groups compared with DIR group and all these findings were statistically significant.

Li *et al*. investigated effects of sevoflorane on lung IR damage [14]. Besides histopathological evaluations, Pco2, dry/wet lung ratio and permeability outcomes were compared and protective effects of sevoflorane on the lungs in IR damage were demonstrated.

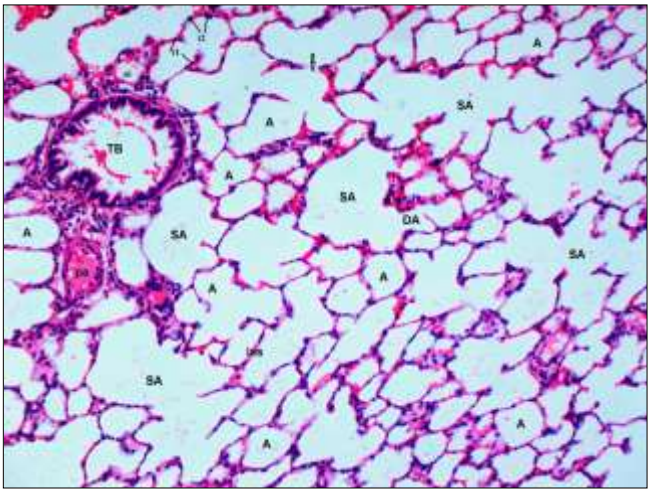
Similarly Liu *et al*. compared pulmonary vascular resistance, microvascular permeability, wet/dry lung rations and the changes in parameters such as LDH, NO and TNF α between the groups and as a result, Liu *et al*. also reported that administration of sevoflurane before ischemia decreased I/R lung in the lungs [7].

Otsuki *et al*. showed that sevoflorane decreases I/R damage in the lungs by making changes in oxygen values (Po2/Fio2) and miRNA expression [15].

In another study Luo *et al*. showed that sevoflorane exerts its effect by decreasing mast cell activation and oxidative damage in the indirect lung damage occurring after intestinal I/R damage [16].

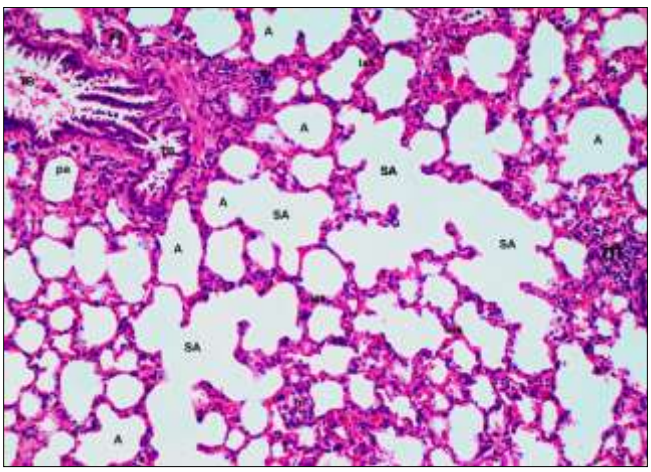
5.Conclusion

In conclusion; sevoflorane and desflorane have positive effects on MDA, SOD and LTIS parameters in I/R damage on the distant organ lungs. It could be considered that indirect lung injury occurring is thus decreased.



(A: alveolus, TB:terminal bronchiole, SA: saccus alveolaris, DA: ductus alveolaris, ias: interalveolar septum, t1: type 1 alveolar cell nucleus, t2: type 2 alveolar cell nucleus, interalveolar kohn gaps, ni: neutrophile infiltration)

Fig 1: Normal-structural lung tissue parenchyma in the control group, HEx100



(A: alveole, TB:terminal bronchiole, SA: saccus alveolaris, pa: blood vessel(branches of pulmonary artery), ias: interalveolar septum, ni: neutrophile infiltration)

Fig 2: Mild neutrophilic infiltration and increased alveolar wall thickness in DC group, HEx100

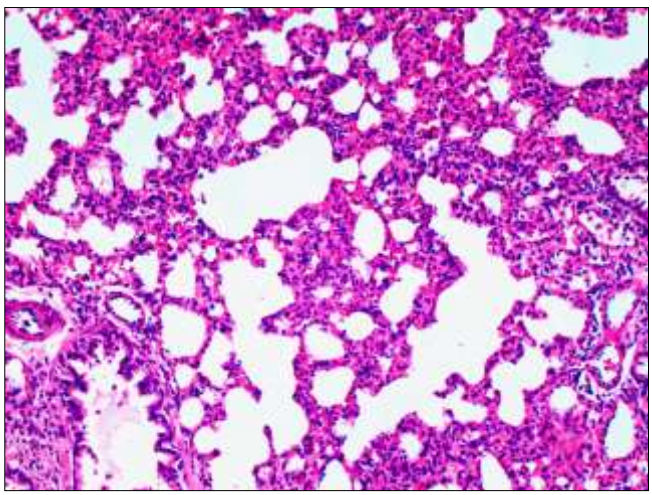
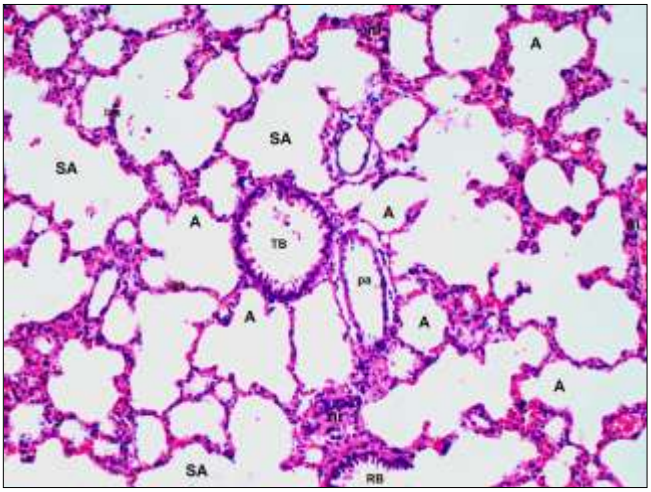
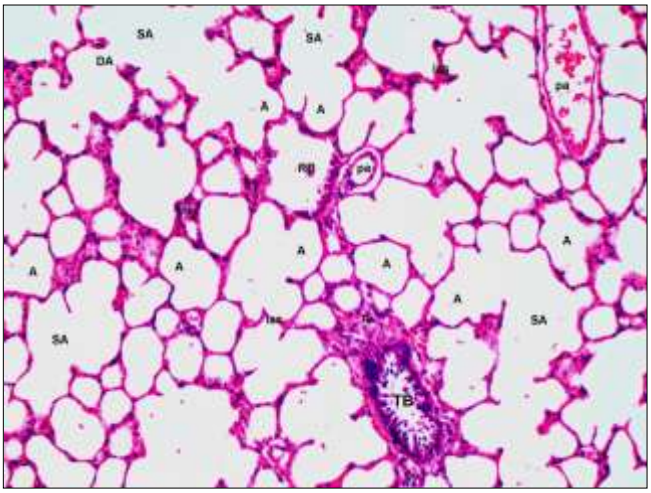


Fig 3: Severe neutrophilic infiltration and increased alveolar wall thickness in DIR group, HEx100



(A: alveoles, TB:terminal bronchiole, RB: respiratuar bronchiole, SA: saccus alveolaris, pa: blood vessel(branches of pulmonary artery), ias: interalveolar septum, ni: neutrophile infiltration)

Fig 4: Mild neutrophilic infiltration and increased alveolar wall thickness in DIRD group, HEx100



(A: alveoles, TB:terminal bronchiole, RB: respiratuar bronchiole, SA: saccus alveolaris, pa: blood vessel (branches of pulmonary artery), ias: interalveolar septum, ni: neutrophile infiltration)

Fig 5: Mild neutrophilic infiltration and increased alveolar wall thickness in DIRD group, HEx100

Table 1: Histopathological findings of the lung tissue [Mean ± SE]

	Group C (n=6)	Grou DC (n=6)	Grou DIR (n=6)	Group DIRD (n=6)	Group DIRS (n=6)	P**
Neutrophile infiltration/aggregation	0.67±0.21*	1.17±0.17*	2.67±0.21	1.50±0.34	1.50±0.34	<0.0001
Alveolar wall thickening	0.17±0.17*	0.67±0.21*	2.33±0.21&	1.50±0.43*,&	1.50±0.34*,&	<0.0001
Score	0.83±0.31*	1.83±0.17*	5.00±0.36&	3.00±0.37*,&	3.00±0.63*,&	<0.0001

p**: Statistical significance was set at a p value < 0.05 for Kruskal-Wallis test
* p<0.05: When compared with Group DIR
& p<0.05: When compared with Group C

Table 2: Oxidant status parameters of rat lung tissue [Mean ± SE]

	Group C (n=6)	Group DC (n=6)	Group DIR (n=6)	Group DIRD (n=6	Group DIRS (n=6)	P**
MDA (nmol/mg prot)	0,81±0,32*	1,25±0,39	1,61±0,27	0,73±0,23*	0,73±0,23*	<0,0001
SOD (IU/mg prot)	59,83±15,70*	151,98±40,07*	444,67±96,13	147,28±23,32*	125,30±32,92*	<0,0001

p**: Statistical significance was set at a p value < 0.05 for Kruskal-Wallis test * p<0.05: When compared with Group DIR

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