

## A comparison of the effects of desflurane and isoflurane on rat pulmonary parenchyme histopathology and malondialdehyde levels

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### Abstract

**Objective:** To evaluate the lipid peroxidation and pulmonary histopathology after desflurane and isoflurane anaesthesia in rats.

**Methods:** The study was conducted in the faculty of Veterinary Medicine Animal Laboratories, Ankara University, between January and December 2009. Twenty-four Wistar-Albino rats were studied and classified randomly into three equal groups. The control group (n=8) was made to inhale 50% O<sub>2</sub> for 60 minutes; the isoflurane group (n=8) received 50% O<sub>2</sub>+1.2% isoflurane for 60 minutes; and the desflurane group (n=8) was given 50% O<sub>2</sub>+6% desflurane, again for 60 minutes. As the sham group, one rat was sacrificed via intracardiac blood aspiration. Rat pulmonary tissue parenchyma samples were evaluated for peribronchial inflammatory infiltration, alveolar septal infiltration, alveolar oedema, exudation, alveolar histiocyte and tissue malondialdehyde levels.

**Results:** When compared with the control group, peribronchial inflammatory infiltration levels were found to be considerably high in the desflurane and isoflurane groups (p= 0.0031). In addition, the alveolar hystiocytes were much higher in the desflurane group than in the control group (p<0.05). Tissue malondialdehyde levels were found to be significantly higher in both groups than in the control group.

**Conclusion:** Desflurane significantly increased pulmonary inflammation more than isoflurane in rat pulmonary parenchyma that indicates an inflammatory response. Besides, it was determined that the significantly higher malondialdehyde levels in both the desflurane and the isoflurane groups resulted in an increase in the membrane lipid peroxidation via volatile anaesthetics.

**Keywords:** Desflurane, Isoflurane, Volatile anaesthetics. (JPMA 62: 1174; 2012)

### Introduction

Chemicals used for general anaesthesia can be described as toxic medicine. The toxicity created by such chemicals is within certain levels and is mainly reversible. It is known that inhalation anaesthetics specially lead to an increase in the release of proinflammatory cytokine and these cytokines increase the influx of neutrophils via alveolar macrophages.<sup>1</sup> Besides, inhalation anaesthetics influence alveolar macrophages which have a very significant role in the defense against post-operative pulmonary complications. It has been reported that such effect on the lungs created by inhalation anaesthetics is usually sub-clinical while contributing to the increase in post-operative pulmonary complications.<sup>2,3</sup>

In this study, the aim was to compare the effects of desflurane and isoflurane anaesthesia in the rat pulmonary tissue on lipid peroxidation and pulmonary histopathology.

### Material and Methods

The study was conducted in the faculty of Veterinary

Medicine Animal Laboratories, Ankara University, between January and December 2009. In the study, irrespective of gender difference, 24 Wistar-Albino rats, weighing between 190 and 315gr, were included. The rats were kept in an environment where they were provided with their required 12-hour daylight and 12-hour darkness periods at a 20-24°C degrees room temperature, and at 65% to 70% humidity levels, without any restriction on their needs for nutrition and liquids. The rats were divided into three groups of 8 rats each: the control group inhaled 50% oxygen for 60 minutes; the isoflurane group inhaled 50% oxygen + 1.2% isoflurane for 60 minutes; and the desflurane group inhaled 50% oxygen + 6% desflurane for 60 minutes.

Before the process, a plastic box 80x40x15 cm in size was formed, and soda-lime, a carbondioxide absorbant, was poured over the lower part of the box. The two sides of the box were covered with cotton paeds, and a heater and mercury thermometer were placed into the box to regulate the temperature. A 2cm diameter hole was perforated on one side of the box, and with the help of the anaesthesia

machine prepared for the purpose, the rats were given 50% oxygen, 6% desflurane and 1.2% isoflurane through the hole. Besides, a capnograph was attached to the side of the pipe, connecting the anaesthesia machine to the box and providing the passage of gas. Thus, the carbon dioxide, desflurane and isoflurane levels of the environment were followed. At the end of the 60-minute process, the rats which survived, reached the sufficient oxygen levels, and came to the supine position were included in the rest of the study. The sufficient anaesthesia levels were reached in all the rats and there was no exitus. The rats in the control group were sacrificed with intracardiac blood aspiration. One rat - sham group - which was kept in an environment where it was provided with its required 12-hour daylight and 12-hour darkness periods, at a 20-24°C degrees room temperature, and at 65% to 70% humidity levels, without any restriction on its needs for nutrition and liquids, and without any special treatment was sacrificed with intracardiac blood aspiration.

The rats in the other groups under anaesthesia were taken out of the box and their thorax surface was shaved and cleaned with a 10% povidon iod solution. Their lungs were totally exposed by entering the thorax from the mid-line incision. Some of the tissues exerted were put into a balanced formaldehyde solution for pathological examination. The remaining tissues were immediately frozen with liquid nitrogen and preserved at -80°C degrees for the evaluation of malondialdehyde (MDA) levels.

In a 10% balanced formaline solution, fixation was applied to the lung tissues for 48 hours. From all the lobes of each lung, a sample that included lobar bronchus was taken. Paraffin blocks were prepared after routine tissue evaluation. Then 5µ thick cross-sections were painted with haemotoxylin-eosin and the microscope slides were evaluated as single-blind by the same person carrying out the process.

MDA levels as products of lipid peroxidation were determined with Thiobarbituric acid (TBA) test as well as

spectrophotometrically. In doing so 0.67% TBA, 1% phosphoric acid, n-butanol, and 1.15% cold potassium chloride (KCI) were used as reactives. After that 0.5 ml from the 10% tissue homogenates in the 1.15% cold KCI was taken and was mixed with a solution consisting of 3 ml 1% phosphoric acid and 1 ml 0.67% TBA. Afterwards, the mixture was kept in boiled water for 45 minutes. The pipes were cooled down, 4ml n-butanol was added to the mixture, and after being centrifuged at a rotation speed of 5000 for 5 minutes, the n-butanol phase on the upper part of the tube was spectrophotometrically read at the level 535-520 nm. The difference between the two absorbants was used as nmol/g MDA level.

The study was approved by the Ethical Committee of Ankara University Veterinary Faculty and the Education Plan - Coordination Committee of Turkiye Yuksek Ihtisas Research and Education Hospital, Ankara/Turkiye.

The data gathered at the end of the study was analysed using SPSS 10. On the crosstabs,  $\chi^2$  and MDA levels were compared with each other with Analysis of Variance (ANOVA) and Kruskal-Wallis tests in all the three groups. After the difference in values was determined, it was assessed as to which couple had created this difference via the post-hoc multiple comparison test. For all tests,  $p < 0.05$  was accepted as statistically significant. It was evaluated with Spearman's correlation test whether there was a correlation between the pathological parameters and MDA levels.

## Results

All cross-sections for every part of the lungs were examined and their parameters were evaluated (Table-1).

There was no statistically significant difference between the groups in terms of the rats' body weights (Table-2).

Peribronchial inflammatory infiltration was observed in all groups when they were compared with normal lung parenchyme tissue (Figure-1-A, 1-B). In the

**Table-1: Pathological evaluation of the lungs.**

Pathological evaluation	Grade 0	Grade 1	Grade 2	Grade 3
Peribronchial Inflammatory cell infiltration	None	Prominent germinal center in lymphoid follicule	Prominent germinal center in lymphoid follicule and lymphocytic infiltration between follicule	Confluent band like peribronchial Inflammatory cell infiltration
Alveolar septal infiltration	None	Focal, minimal	Prominent infiltration in alveoli wall	Infiltration in alveolar walls with narrow lumen
Alveolar oedema	None	Focal minimal	In alveoli lumen	In lobule
Alveolar exudate	None	Focal minimal	In alveoli lumen	In lobule
Alveolar macrophage	None	A few alveoles rarely	Clustering in alveoli lumen	Macrophage cluster that fills alveolar lumen
Granuloma	None	Rare microgranuloma	Rare well formed granulomas	Confluent granulomas
Necrosis	None	Focal, a few apoptosis	Focal, multiple necrosis foci	Focal necrosis which impairs the lobular and bronchiolar tissue

**Table-2: Body weights of the rats, the changes in lung histopathology and tissue MDA levels.**

Parameter	Control	Isoflurane	Desflurane
Body weights (gr)	254.5 ± 55.8	247.7 ± 43.4	248.7 ± 55.2
Peribronchial Inflammatory infiltration degree	0.75 ± 0.71	1.37 ± 0.74	1.75 ± 1.04*
Alveolar septal infiltration	1.25 ± 0.46	2 ± 0.00	2.13 ± 0.99
Alveolar oedema	1.25 ± 0.46	1.50 ± 0.53	1.75 ± 0.89
Alveolar macrophage	1.25 ± 0.46	1.63 ± 0.52	2.13 ± 1.13*
MDA level	15.56 ± 4.25	25.21 ± 6.99	26.70 ± 5.63

\*p <0,05

MDA: Malodialdehyde.

desflurane group, peribronchial inflammatory infiltration was found to be considerably higher than the one in the control group (p= 0.0031). Alveolar septal infiltration was seen at various levels in all the rats in all the three groups (Figure 1-C). In 4 (50%) rats in the desflurane group, inflammatory infiltration was observed at such an intensity to constrict the lumen in the alveolar walls. No statistically significant difference was seen in the alveolar septal infiltration intensity levels among the groups.

When compared with the control group, there was no statistically significant difference in the alveolar oedema intensity levels of the two groups. Alveolar oedema was

observed in the lobule in two rats in the desflurane group (Figure-1-D).

Alveolar exudate was observed in the alveoli lumen in 1 (12.5%) rat in the desflurane group (Figure-1-E). In the other groups, no alveolar exudate image was present. There was no statistically significant difference between the groups in terms of alveolar exudate ( p>0.05).

Alveolar histiocytes were found to be statistically considerably higher in the desflurane group when compared with the control group (p<0.05) (Figure 1-F). In 4 (50%) rats in the desflurane group, a set of histiocytes filling the alveoli lumen was present.

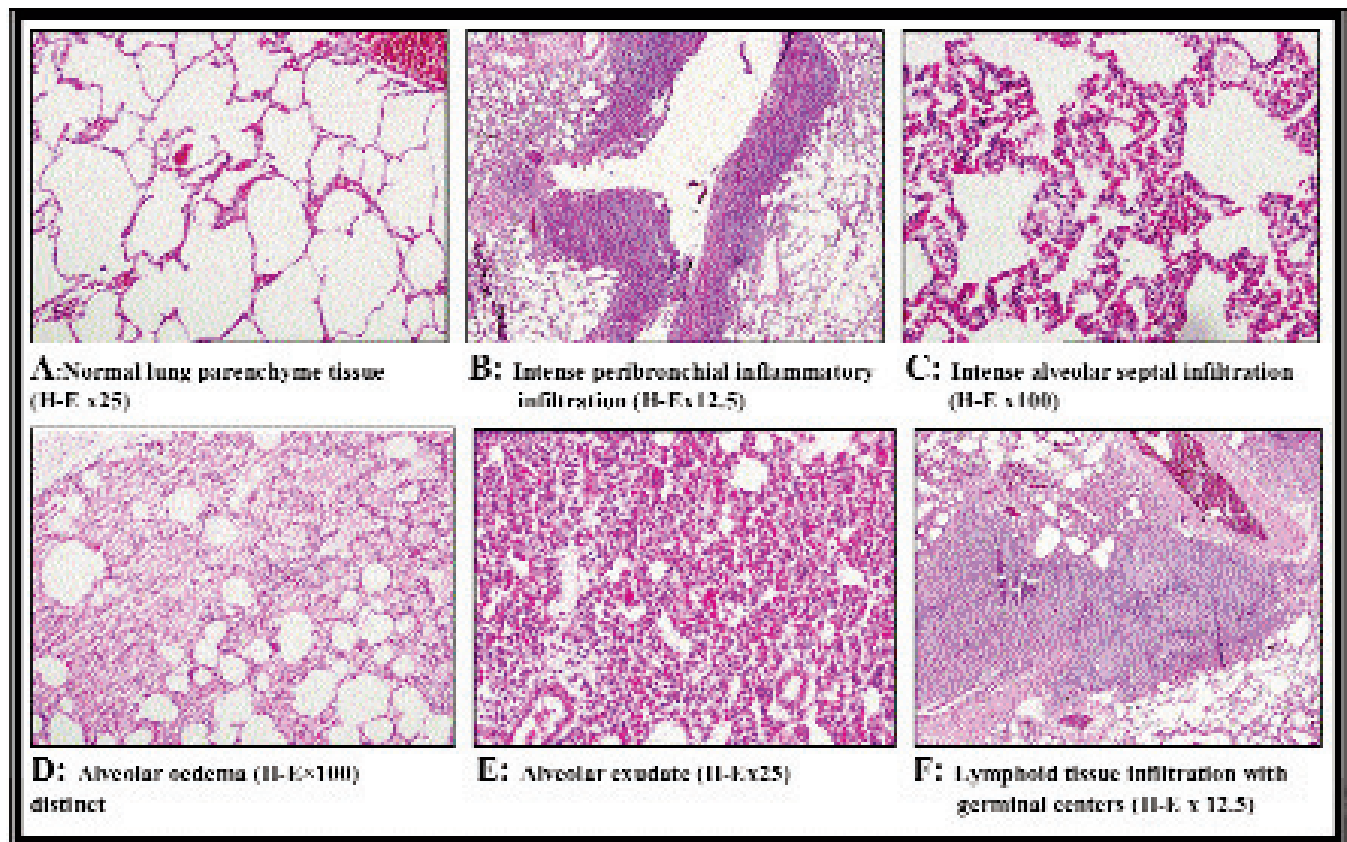


Figure: Histopathological views of pulmonary tissue.

In none of the groups, granuloma and necrosis were observed.

Tissue MDA levels were found to be statistically significantly higher in the two groups than in the control group ( $p < 0.05$ ).

## Discussion

Pulmonary complications have been frequently encountered after anaesthesia and surgery. Although the effect of anaesthetics on pulmonary complications are not known definitively, it has been thought that pulmonary defense system is affected by anaesthetics.<sup>1</sup>

In the studies conducted so far, it has been established that volatile anaesthetics increase proinflammatory cytokine release and macrophage aggregation,<sup>2,3</sup> and that the volatile anaesthetics applied after experimental aspiration increase the degree of lung damage and the rate of mortality.<sup>4</sup>

A study analysed the effects of sevoflurane on the ultra-structure of pig lung and they showed that sevoflurane had no destructive effect on the ultra-structure of the lung after comparing them with the groups to which they gave thiopental after a six-hour 1.5 Minimal anaesthetic concentration (MAC) sevoflurane.<sup>5</sup> A similar study revealed that sevoflurane leads to a moderate inflammatory mediator increase in the lungs.<sup>6</sup> Analysing the wholeness of the alveoli-capillary barrier in patients who received halothane and isoflurane after anaesthesia by Technetium-99 m Hexamethylpropylene Amine Oxime (Tc-99 m HMPAO), another study proved that in the early post-operative period, these two agents lead to a temporary damage and develop sub-clinical symptoms.<sup>7</sup>

In our study, it has been observed that the 6% desflurane and 1.2% isoflurane given to the rats for a one-hour period resulted in peribronchial inflammatory infiltration in all the groups. While peribronchial inflammatory infiltration was found to be higher in the desflurane group than in the control group, no significant increase was observed in the isoflurane group. As for alveolar septal infiltration, it was similar in all groups although seen in different levels in the three groups and causing inflammatory infiltration at an intensity to constrict the alveoli lumen in 4 (50%) rats in the desflurane group. In neither of the two groups, alveolar exudate and oedema revealed a significant difference compared to the control group. However, the shortness of the duration of anaesthesia might have been an impeding factor in the observation of these effects. The significant increase in the level of peribronchial infiltration in the desflurane group seems to be in support of the damage on the lung tissue caused by other volatile anaesthetics.

The other parameter taken into consideration in observing lung tissue parenchyma was macrophage. Alveolar macrophage plays an important role in the antibacterial defence of the lung, and their contributions to the persistence of the normal functions and the sterility of the lung are important.<sup>1</sup> In the current study, alveolar macrophages were evaluated according to the aggregations they caused in the alveoli walls found in the lung alveolar lumen. Various in vitro animal experiments examining the immunologic defence system in the lung have also shown that volatile agents suppress the cytotoxic and phagocytic responses of alveolar macrophages.<sup>8,9</sup> Distinct changes in the tissue rates in the bronchoalveolar lavage (BAL) liquid depend on the toxic effects of anaesthesia and macrophages on alveolar macrophages.<sup>10</sup> In the applications of anaesthesia, intubation, positive pressure ventilation, anaesthetic gases, increased oxygen pressure, and the release of inflammatory mediators in the airway and pulmonary tissue might cause a delay in the immune response, depending on surgery and increased oxygen pressure.<sup>11</sup> In our study, the absence of intubation, surgery and positive pressure ventilation, and the analysis of tissue macrophages instead of BAL have caused the results to remain unaffected by these changes.

A study detected a decrease in the liveliness and an increase in the alveolar macrophage aggregations of 41 patients who underwent abdominal surgery and were given isoflurane anaesthesia.<sup>3</sup> Yet it was seen that this was a reversible effect. Another study showed that while neutrophil migration and pulmonary macrophage aggregation in isoflurane and propofol anaesthesia increase in time, phagocytic and microbicidal activities show a decrease. It concluded that this has been more distinct in isoflurane.<sup>11</sup> Another study revealed that isoflurane and halothane increase the number of immature alveolar macrophage in the BAL liquid, and that only these are immunely non-functional.<sup>12</sup> In our study, macrophages in the lung tissue parenchyma were evaluated according to the aggregation intensity they caused in the alveoli lumen, and were found to be significantly higher in the the group given desflurane than in the control group.

A study with 30 pigs found the values of the MDA levels in the plasma and BAL liquid to be significantly higher in cases under the influence of desflurane anaesthesia than in those under propofol and sevoflurane anaesthesia.<sup>2</sup> Another study with 20 patients under the influence of sevoflurane and desflurane anaesthesia during laparoscopic surgery, showed that the MDA levels taken from the BAL liquids of these patients were significantly higher in the desflurane group than in the sevoflurane group.<sup>13</sup> In our study, a significant increase was observed in the lung tissue MDA levels that were the products of lipid peroxidation in

both groups of rats given isoflurane and desflurane. The studies cited above show parallelism with the MDA increase in our desflurane and isoflurane groups, and seem to be in support of the view that volatile anaesthetics cause free radical damage.

### Conclusion

The study showed that, compared with isoflurane, desflurane led to a more significant inflammatory response in lung tissue parancyhma, and that the high levels of MDA levels in desflurane and isoflurane groups, in contrast to the control group, indicated that volatile anaesthetics cause an increase in membrane lipid peroxidation.

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### References

1. Kotani N, Lin CY, Wang JS, Gurley JM, Tolin FP, Michelassi F, et al. Loss of alveolar macrophages during anesthesia and operation in humans. *Anesth Analg* 1995; 81: 1255-62.
  2. Allaouchiche B, Debon R, Goudable J, Chassard D, Duflo F. Oxidative stress status during exposure to propofol, sevoflurane and desflurane. *Anesth Analg* 2001; 93: 981-5.
  3. Kotani N, Hashimoto H, Sessler DI, Yasuda T, Ebina T, Muraoka M, et al. Expression of genes for proinflammatory cytokines in alveolar macrophages during propofol and isoflurane anesthesia. *Anesth Analg* 1999; 89: 1250-6.
  4. Molliex S, Crestani B, Dureuil B, Rolland C, Aubier M, Desmonts JM. Differential effects of isoflurane and i.v. anaesthetic agents on metabolism of alveolar type II cells. *Br J Anaesth* 1999; 82: 767-9.
  5. Takala RS, Soukka HR, Salo MS, Kirvela OA, Kaapa PO, Rajamaki AA, et al. Pulmonary inflammatory mediators after sevoflurane and thiopentone anaesthesia in pigs. *Acta Anaesthesiol Scand* 2004; 48: 40-5.
  6. Takala RS, Soukka HR, Kirvela OA, Kujari HP, Pelliniemi LJ, Kaapa PO, et al. Alveolar integrity and ultrastructure in pigs remain undamaged after exposure to sevoflurane. *Acta Anaesthesiol Scand* 2002; 46: 1137-43.
  7. Hung CJ, Liu FY, Shaiu YC, Kao A, Lin CC, Lee CC. Assessing transient pulmonary injury induced by volatile anesthetics by increased lung uptake of technetium-99m hexamethylpropylene amine oxime. *Lung* 2003; 181: 1-7.
  8. Sun SS, Hsieh JF, Tsai SC, Ho YJ, Kao CH. Transient increase in alveolar epithelial permeability induced by volatile anesthesia with isoflurane. *Lung* 2000; 178: 129-35.
  9. Giraud O, Molliex S, Rolland C, Lecon-Malas V, Desmonts JM, Aubier M, et al. Halogenated anesthetics reduce interleukin-1beta-induced cytokine secretion by rat alveolar type II cells in primary culture. *Anesthesiology* 2003; 98: 74-81.
  10. Cherniack RM, Colby TV, Flint A, Thurlbeck WM, Waldron J, Ackerson L, et al. Quantitative assessment of lung pathology in idiopathic pulmonary fibrosis. The BAL Cooperative Group Steering Committee. *Am Rev Respir Dis* 1991; 144: 892-900.
  11. Kotani N, Hashimoto H, Sessler DI, Kikuchi A, Suzuki A, Takahashi S, et al. Intraoperative modulation of alveolar macrophage function during isoflurane and propofol anesthesia. *Anesthesiology* 1998; 89: 1125-32.
  12. Yasith H, Durak P, Kobat I, Aydog G, Demirbag AE, Bahadir G, et al. Bronchoalveolar lavage (BAL) during sevoflurane, isoflurane and halothane anaesthesia. *Acta Anaesthesiol Ital* 1998; 49: 269-73.
  13. Koksak GM, Sayilgan C, Aydin S, Uzun H, Oz H. The effects of sevoflurane and desflurane on lipid peroxidation during laparoscopic cholecystectomy. *Eur J Anaesthesiol* 2004; 21: 217-20.
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