

## Effects of Sevoflurane and Desflurane on Oxidant/Antioxidant Status of Young Versus Old Rat Liver Tissues

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**Abstract:** Anesthetic agents modulate on oxidant/antioxidant activity. This study aimed to evaluate the effects of desflurane and sevoflurane anesthesia on the oxidant/antioxidant activity in the liver of young and aged rats. The study involved 60 male Wistar Albino Rats. Rats, which 5-6 months of age deemed as young (Group Y, n = 30) and 10-11 months of age deemed as old (Group O, n = 30). The weight range of the rats was 270-350 g. The groups of rats were randomly divided into 3 groups as the control group [Group Y<sub>C</sub> (Young Control 100% O<sub>2</sub>; n = 10) and Group O<sub>C</sub> (Old Control 100% O<sub>2</sub>; n = 10)], desflurane group [Group Y<sub>D</sub> (Young Desflurane; 6% Desflurane in 100% O<sub>2</sub>; n = 10)] and Group O<sub>D</sub> (Old Desflurane; 6% Desflurane in 100% O<sub>2</sub>; n = 10)] and sevoflurane group [Group Y<sub>S</sub> (Young Sevoflurane; 2% Sevoflurane in 100% O<sub>2</sub>; n = 10) and Group O<sub>S</sub> (Old Sevoflurane; 2% Sevoflurane in 100% O<sub>2</sub>; n = 10)]. The rats placed into a transparent plastic cage. The rats were exposed to different anesthetic agents or oxygen for 2 h by the use of half open Anesthesia System (AMS, Senior 425), while rats' simultaneous normal breathing was maintained. At the end of the exposure, they were administered a high dose of ketamine and the livers of the animals were surgically removed. SOD, GST and NOS activities were determined and levels of oxidative stress was monitored by measuring TBARS via levels of MDA in the liver. Desflurane induce oxidative stress in both young and old rats, with higher levels in old rats. However, sevoflurane did not cause oxidative stress in young rats. Sevoflurane increased the oxidative stress in the old rats based on SOD and TBARS levels, while it maintained GST activity and decreased NOS activity. However, further studies are needed.

**Key words:** Oxidative stress, desflurane, sevoflurane, rat, age

### INTRODUCTION

Oxidative Stress (OS) arises due to an imbalance between the production and elimination of free oxygen radicals that are generated during aerobic metabolism and consumed endogenously. Oxidative stress is very common in various pathologies such as respiratory distress syndrome, atherosclerosis, chronic renal failure, rheumatoid arthritis, diabetes, sepsis and Alzheimer disease. Ischemia, inflammation, aromatic hydrocarbons, strenuous exercise, antineoplastic agents, antibiotics, as well as environmental factors such as radiation, cigarette smoke and air pollution, which affect the level of free

oxygen radicals by converting into radicals either directly or during intracellular metabolism or detoxification. Aging intensifies the effects of OS producing factors as well as being an OS source itself (Sies, 1997; Tan *et al.*, 1998; Kim *et al.*, 2002; Wilson *et al.*, 2002; Dalle-Donne *et al.*, 2003; Sullivan *et al.*, 2004).

The role of anesthetic agents in OS production or protection against the harmful effects of free oxygen radicals has been a topic of significant studies (Durak *et al.*, 1996a; Durak *et al.*, 1997; Durak *et al.*, 1999; Allaouchiche *et al.*, 2001; Wilson *et al.*, 2002). The studies have shown that halothane or desflurane have negative effects on the antioxidant system of various

tissues (Durak *et al.*, 1996a, 1997; Allaouchiche *et al.*, 2001), while propofol shows antioxidant characteristics (Allaouchiche *et al.*, 2001; Runzer *et al.*, 2002). In several studies, lowered, increased or unchanged OS were observed (Allan *et al.*, 1987; Xu *et al.*, 1998; Durak *et al.*, 1999; Allaouchiche *et al.*, 2001; Schneemilch *et al.*, 2005). In this respect, literature reveals conflicting results on the effects of these anesthetic agents.

There are many reports on the use of anesthetic agents that are rapidly eliminated from the body and/or sevoflurane. However, few studies have been performed on age-dependent effects of desflurane. Thus, this study aimed to compare the oxidant/antioxidant activity in the liver tissue samples of young and old rats that were exposed to sevoflurane or desflurane anesthetics for 2 h.

In aerobic organisms, it is possible to evaluate OS by studying thiobarbituric acid-reactive substances (TBARS) and nitric oxide synthase (NOS) levels and antioxidant defense systems based on superoxide levels such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), glutathione-S transferase (GST), catalase (CAT) and glutathione reductase (Richard *et al.*, 1992; Sessa, 1994; Bezerra *et al.*, 2004; Prabhu *et al.*, 2004). Accordingly, SOD, GST, NOS and TBARS levels were studied in the liver tissue samples of the rats to determine the presence of oxidative stress and antioxidant activity.

## MATERIALS AND METHODS

This study was performed at Erciyes University Experimental and Clinical Research Center (DEKAM) upon experimental animals' ethics committee approval of Gazi University.

The study involved 60 male Wistar Albino Rats. Rats, which 5-6 months of age deemed as young (Group Y, n = 30) and 10-11 months of age deemed as old (Group O, n = 30).

The weight range of the rats were 270-350 g. The groups of rats were randomly divided into 3 groups as the control group [Group Y<sub>c</sub> (Young Control 100% O<sub>2</sub>; n = 10) and Group O<sub>c</sub> (Old Control 100% O<sub>2</sub>; n = 10)], desflurane group [Group Y<sub>D</sub> (Young Desflurane; 6% Desflurane in 100% O<sub>2</sub>; n = 10)] and Group O<sub>D</sub> (Old Desflurane; 6% Desflurane in 100% O<sub>2</sub>; n = 10)] and sevoflurane group [Group Y<sub>S</sub> (Young Sevoflurane; 2% Sevoflurane in 100% O<sub>2</sub>; n = 10) and Group O<sub>S</sub> (Old Sevoflurane; 2% Sevoflurane in 100% O<sub>2</sub>; n = 10)].

Before the experiment, the rats were kept in a medium of 20-24°C with a rhythm of 12 h daylight and 12 h darkness. Food was accessible for the rats until 2 h before anesthesia application.

The rats were placed in a transparent plastic cage of 40×40×70 cm in size. An anesthesia machine with a half-open system (AMS, Senior 425) was connected to the plastic cage with static hoses. Anesthetic gas vaporizers were calibrated. Then, setting the minimum alveolar concentration at (MAC) 1, in the inspirium, at concentrations of 6% desflurane, 2% sevoflurane in 100% O<sub>2</sub> or only 100% O<sub>2</sub> were given to the groups for 2 h (Hirai, 1987). At the end of inhalation anesthesia procedure, the rats were intraperitoneally administered 50 mg kg<sup>-1</sup> ketamine and then the abdomens of the rats were opened. Upon intraabdominal blood sampling, the rats were euthanized. Preserving the tissue integrity, liver biopsy samples were obtained paying attention not to traumatize.

**Biochemical analysis:** The liver tissues were first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizer. Measurements on cell contest require initial preparation of the tissues. Preparation may involve grinding of the tissue in a ground glass tissue blender using a rotor driven by a simple electric motor. The homogenizer as a tissue blender similar to the typical kitchen blender is used to emulsify and pulverize the tissue (Heidolph Instruments GMBH and CO KG DiAx 900 Germany®) at 1000 U for about 3 min. After centrifugation at 10 000 g for about 60 min, the upper clear layer was taken. In this fraction, total nitric oxide synthase (T-NOS) activity and nitric oxide (NO) pool (NO• +NO<sub>2</sub>) were measured as described, respectively (Ignarro *et al.*, 1987; Bucala *et al.*, 1991). NOS activity method was based on the diazotization of sulfanilic acid by nitric oxide at acid pH and subsequent coupling to N-(1-naphthyl-ethylene diamine), which is the modification of a previous study (Ignarro *et al.*, 1987; Durak *et al.*, 2001). The analysis scheme of the NOS activity measurement method has been described in a previous study (Durak *et al.*, 2001). Measurement of the NO pool (mainly consisting of NO• +NO<sub>2</sub>) is also based on the same chemical reaction, in which to a greater extent nitric oxide (NO•) and to a lesser extent nitrite anion (NO<sub>2</sub><sup>-</sup>), but not nitrate anion (NO<sub>3</sub><sup>-</sup>), give a diazotization reaction with sulfanilic acid. The absorbance of complexone formed with N-(1-naphthyl-ethylene diamine) reflects the sum of NO• and NO<sub>2</sub><sup>-</sup> levels in the reaction medium, which is termed 'the NO pool' in the present study. In this method, sodium nitroprusside is used as the chemical standard and the reaction scheme given for the NOS activity measurement, except for the incubation of the sample with arginine, is followed. The thiobarbituric

acid-reactive substances levels were determined in the same supernatant fraction by using the thiobarbituric acid method of Van Ye *et al.* (1993) and were expressed in nmol mg<sup>-1</sup> protein.

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 total superoxide dismutase (T-SOD) and total Glutathione-S Transferase (T-GST). After centrifugation at 10.000 × g for 60 min, the upper clear layer was removed and used for the analyses.

The SOD activity method is based on the measurement of absorbance increase at 560 nm due to reduction of NBT to NBTH<sub>2</sub> (Durak *et al.*, 1996a). One unit of SOD activity was defined as the enzyme protein amount causing 50% inhibition in NBTH<sub>2</sub> reduction rate and the results were expressed in U mg<sup>-1</sup> protein.

The GST activity was assayed using the procedure described by Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate in ultraviolet (UV by sequential addition of 0.1 M phosphate buffer, pH 6.5 (1.5 mL), enzyme preparation as above (0.1 mL), 50 mM of reduced GSH solution in buffer (0.2 mL) and 25 mM CDNB solution in ethanol (0.15 mL) (2 mL final volume of the routine incubation mixture). Enzyme activity was determined by continuously monitoring the change in absorbance at 340 nm for 3 min at 25°C with a Shimadzu UV-1601 spectrophotometer and results were expressed in mIU mg<sup>-1</sup> protein. Protein amounts were measured as described by Lowry *et al.* (1951).

**Statistical analyses:** The statistical analyses were performed with SPSS 12.0 software program and p<0.05 was considered statistically significant. The findings were expressed as mean±standard deviation. The data were evaluated with Kruskal-Wallis variance analysis. The variables with significance were evaluated with Bonferroni corrected Mann-Whitney U test.

**RESULTS**

SOD, GST, NOS and TBARS levels of the groups have been listed in Table 1.

**Effects of aging on SOD, GST, NOS activities:** No significant differences were detected among the SOD, GST, NOS activities and TBARS levels of the young and old control groups. However, SOD, NOS activities and TBARS levels were significantly elevated in the old sevoflurane and desflurane groups versus the controls p<0.05; Fig. 1-4.

Table 1: The oxidant/antioxidant activity levels of the groups

Groups (n = 10)	SOD	GST (n = 10)	NOS (n = 10)	TBARS (n = 10)
Y <sub>C</sub>	5.52±2.35	8.74±1.41	7.10±2.88	0.68±0.27
O <sub>C</sub>	5.80±3.68	9.22±2.30	7.00±3.30	0.80±0.493
Y <sub>D</sub>	12.32±3.39*,**	14.56±0.80*,**	5.23±2.04	1.28±0.48*,**
O <sub>D</sub>	14.84±1.17*,**	15.31±1.69*,**	13.00±3.95*,**	3.25±1.49*,**
Y <sub>S</sub>	7.99±0.82	10.45±1.35	3.13±0.98*,**	0.75±0.31
O <sub>S</sub>	12.91±1.14*,**	9.84±0.84	3.13±0.72*,**	1.61±0.12*,**

Values are mean±SD, \*p<0.05 when compared to group Y<sub>C</sub>, \*\*p<0.05 when compared to group O<sub>C</sub>

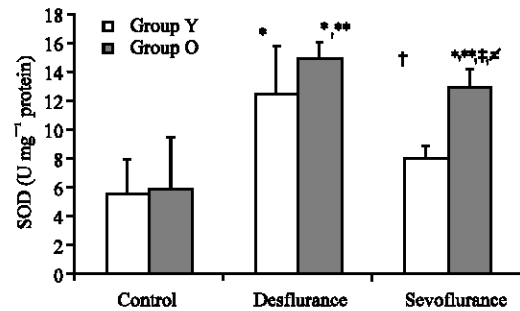


Fig. 1: The SOD levels of the groups. Values are mean±SD. \*p<0.05, Group Y<sub>C</sub>; \*\*p<0.05, Group O<sub>C</sub>; †p<0.05, Group Y<sub>D</sub>; ‡p<0.05, Group O<sub>D</sub>; ≠ when compared to Group Y<sub>S</sub> (Y<sub>C</sub> = Young control; Y<sub>S</sub> = Young sevoflurane; Y<sub>D</sub> = Young desflurane; O<sub>C</sub> = Old control; O<sub>D</sub> = Old desflurane)

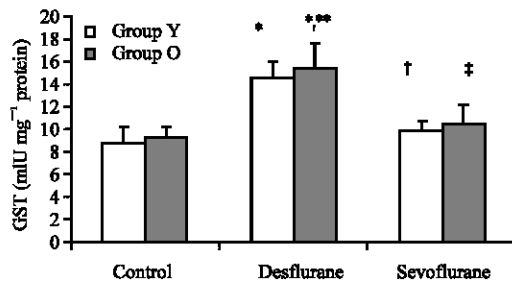


Fig. 2: The GST levels of the groups. Values are mean±SD. \*p<0.05, Group Y<sub>C</sub>; \*\*p<0.05, Group O<sub>C</sub>; †p<0.05, Group Y<sub>D</sub>; ‡p<0.05, Group O<sub>D</sub> (Y<sub>C</sub> = Young control; Y<sub>S</sub> = Young sevoflurane; Y<sub>D</sub> = Young desflurane; O<sub>C</sub> = Old control)

**Effects of sevoflurane on SOD, GST, NOS activities (young rats):** NOS activity was significantly decreased in young sevoflurane group compared to that in the control group p<0.05; Fig. 1-4.

**Effects of sevoflurane on SOD, GST, NOS activities (old rats):** In the old sevoflurane group, NOS activity was significantly reduced, while SOD activity and TBARS levels were significantly elevated p<0.05; Fig. 1-4.

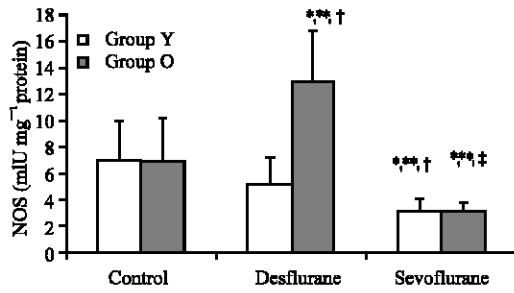


Fig. 3: The NOS levels of the groups. Values are mean±SD \*p<0.05, Group Y<sub>C</sub>; \*\*p<0.05, Group O<sub>C</sub>; †p<0.05, Group Y<sub>D</sub>; ‡p<0.05, Group O<sub>D</sub> (Y<sub>C</sub> = Young control; Y<sub>S</sub> = Young sevoflurane; Y<sub>D</sub> = Young desflurane; O<sub>C</sub> = Old control)

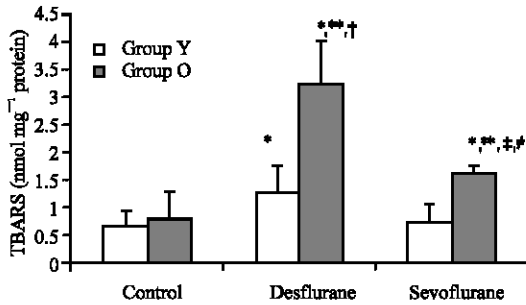


Fig. 4: The TBARS levels of the groups. Values are mean±SD. \*p<0.05, Group Y<sub>C</sub>; \*\*p<0.05, Group O<sub>C</sub>; †p<0.05, Group Y<sub>D</sub>; ‡p<0.05, Group O<sub>D</sub>; # when compared to Group Y<sub>S</sub> (Y<sub>C</sub> = Young control; Y<sub>S</sub> = Young sevoflurane; Y<sub>D</sub> = Young desflurane; O<sub>C</sub> = Old control; O<sub>D</sub> = Old desflurane)

**Effects of desflurane on SOD, GST, NOS activities and OS (young rats):** SOD, GST and NOS activities were significantly elevated in the rats treated with sevoflurane and desflurane in young rats groups versus control p<0.05; Fig 1-4.

**Effects of sevoflurane on SOD, GST, NOS activities (old rats):** In old sevoflurane groups, while SOD, GST activities and TBAS levels were significantly increased, NOS activity was significantly decreased compared to those of the control group p<0.05; Fig. 1-4.

### DISCUSSION

The findings of the study indicated that desflurane treated young and old rat groups had higher SOD, GST and TBARS levels than the control and sevoflurane groups did, clearly suggesting that desflurane tends to create more oxidative stress and increase antioxidant

activity. On the other hand, sevoflurane did not cause OS in the young rats, while it intensified OS and antioxidant SOD activity in the old rats based on their TBARS levels, while maintaining GST activity and suppress NOS activity.

Free oxygen radicals are essential for normal immune defense and metabolic activity. However, in their overproduction or when they could not be eliminated, they cause cellular damage and DNA mutations through chemical modification of cellular protein, carbohydrate, nucleotide and lipids (Tan *et al.*, 1998; Wilson *et al.*, 2002). The condition, which is defined as oxidative stress, increases the enzymes of the antioxidant defense system in the liver tissue (Sies, 1997; Dalle-Donne *et al.*, 2003). Increases in the enzymes may be caused by direct mechanisms that can damage the liver tissue, as well as by hypoxia, chronic liver diseases, viral infections, blood transfusions, septicemia, burns, gestation, nutritional defects, surgical stress, some drugs and anesthetic agents (Allan *et al.*, 1987; Sies, 1997; Schmidt *et al.*, 1999; Wilson *et al.*, 2002; Dalle-Donne *et al.*, 2003; Delogu *et al.*, 2004; Prabhu *et al.*, 2004; Turkan *et al.*, 2005). Anesthetic agents may act in the production of free oxygen radicals by direct effect on the antioxidant system, decreasing the hepatic blood flow, or creating hemodynamic modifications (Lind *et al.*, 1989). Regulation of antioxidant enzyme activity is affected by age and gender (Kang *et al.*, 2005; Lopez-Diazguerrero *et al.*, 2005). Thus, the whole study was performed on the male rats that were not subjected to any other drugs except anesthetic agents and surgical interventions until the blood samples were obtained. Thus, all the other factors that could have any negative effects on the antioxidant system or oxidative stress were eliminated. Nutritional defect was avoided in the rats by free access to food until right before the experiment and the medium of the experiment was not hypoxic during the experiment. All these measures eliminated possible risk factors for development of oxidative stress and except for anesthetic agents and age, all the groups had equivalent conditions. Time is an important factor in maintaining a constant anesthetic concentration in rats (Hirai, 1987). Therefore, anesthetic agent was administered for 2 h.

Certain biochemical tests guide in determining oxidative stress or antioxidant activity in the liver tissue samples. Oxygen radicals produced at the cellular level are detoxified by SOD and the final product is H<sub>2</sub>O<sub>2</sub>, which is also detoxified by intracellular GSHPx enzyme. Glutathione S-transferase is the one of the key enzymes that keeps the cellular membrane intact. GST, which is also, known as non-Se GsHPx, is a part of antioxidant enzymatic system and plays a role in detoxification of lipid hydroxyl,

peroxides (Prabhu *et al.*, 2004). Glutathione S-transferase is localized in centrolobular hepatocytes; therefore, it is much more specific than ALT and AST for hepatic damage (Richard *et al.*, 1992). Glutathione S-transferase is known to be a more important marker for hepatotoxicity associated with volatile anesthetic agents. In the study of Schmidt *et al.* (1998), an elevation in GST levels was observed, while ALT, AST and GGT did not change (Schmidt *et al.*, 1999). Therefore, being a specific marker of hepatotoxicity and one of the cellular antioxidant enzymes, GST is important for free radical metabolism. Malondialdehyde is currently estimated by measurement of TBARS. In our study, TBARS levels were studied as a marker of cellular membrane injury. TBARS is considered as a marker of lipid peroxidation injury via free radicals and it is assessed in studies evaluating the effects of volatile anesthetic agents (Bezerra *et al.*, 2004). NOS level, however, is a marker for assessment of the endothelial function of oxidative stress (Wolin *et al.* 2005). In our study, oxidant/antioxidant effects of anesthetic agents were evaluated based on the levels of TBARS, NOS, GST and SOD in the rat liver tissue samples.

Numerous studies have investigated the effects of anesthetic agents on oxidant/antioxidant system. Some experimental studies have shown the potential antioxidant effects of intravenous anesthetic agents such as ketamine and propofol (Runzer *et al.*, 2002; Kohjitani *et al.*, 2003). Midazolam, however, has been reported to decrease NOS activity (Kohjitani *et al.*, 2003). Some studies have shown that halothane has negative effects on the antioxidant system of various tissues and toxic effects on various organs following ischemia or reperfusion (Allan *et al.*, 1987; Lind *et al.*, 1989; Durak *et al.*, 1996b, 1997). In other studies with different methodologies, sevoflurane either produced oxidative stress or showed antioxidant characteristics (Schlack *et al.*, 1998; Xu *et al.*, 1998; Allaouchiche *et al.*, 2001; Bezerra *et al.*, 2004). Bezerra *et al.* (2004) used isoniazid+sevoflurane or sevoflurane alone in 42 male Wistar rats and detected elevated TBARS levels, with significantly increased levels in the rats that were exposed to isoniazid pretreatment. Allaouchiche *et al.* (2001) studied the effects of sevoflurane and desflurane in swine and determined that those that received sevoflurane had less severe local and ischemic stress than those that received desflurane. In our study, desflurane increased oxidative stress, while sevoflurane did not cause oxidative stress in young rats.

Literature does not reveal any studies on the age dependent effects of inhalation agents on oxidative stress. However, continuous exposure to oxidative stress is known to cause aging and old age in itself

is a factor that increases oxidative stress (Sies, 1997; Dalle-Donne *et al.*, 2003; Kang *et al.*, 2005; Lopez-Diazguerrero *et al.*, 2005). López-Diazguerrero *et al.* (2005) found that DNA damage inflicted by carbon tetrachloride, an oxidative stressor, was more severe in old rats than in young rats. Kang *et al.* (1998) studied 36 young (3 weeks old) and old (16 weeks of age) female rats and found that antioxidant treatment was more effective in decreasing TBARS concentration in adult rats than it was in young rats. In addition, they reported that GST levels were lower in young rats than in adult rats. These findings clearly show the effect of age on antioxidant treatment. In our study, desflurane increased the oxidative stress, with higher levels in old rats than in young rats. It was also determined that with sevoflurane use, oxidative balance was better maintained in young rats than in old rats; the GTS levels of young rats were significantly higher than those of the controls, while their NOS levels had decreased.

## CONCLUSION

Desflurane increased the oxidative stress in young and old rats, while sevoflurane did not activate oxidative stress in young rats. Further investigations are needed on the effects of sevoflurane on old rats.

## REFERENCES

- Allan, L.G., A.J. Hussey, J. Howie, G.J. Beckett, A.F. Smith, J.D. Hayes and G.B. Drummond, 1987. Hepatic glutathione S-transferase release after halothane anesthesia: Open randomised comparison with isoflurane. *Lancet*, 1: 771-774.
- Allaouchiche, B., R. Debon, J. Goudable, D. Chassard and F. Duflo, 2001. Oxidative stress status during exposure to propofol, sevoflurane and desflurane. *Anesth. Analg.*, 93: 981-985.
- Bezerra, F., A.A. Rezende, S.J. Rodrigues and M.G. Almedia, 2004. Thiobarbituric acid reactive substances as an index of lipid peroxidation in sevoflurane-treated rats. *Rev. Bras. Anesthesiol.*, 54: 640-649.
- Bucala, R., K.J. Tracey and A. Cerami, 1991. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J. Clin. Invest.*, 87: 432-438.
- Dalle-Donne, I., R. Rossi, D. Giustarini, A. Milzani and R. Colombo, 2003. Protein carbonyl groups as biomarkers of oxidative stress. *Clin. Chim. Acta*, 329: 23-38.

- Delogu, G., A. Antonucci, S. Moretti, M. Marandola, G. Tellan, M. Signore and G. Famularo, 2004. Oxidative stress and mitochondrial glutathione in human lymphocytes exposed to clinically relevant anesthetic drug concentrations. *J. Clin. Anesth.*, 16: 189-194.
- Durak, I., T. Guven, M. Birey, H.S. Ozturk, O. Kurtipek, M. Yel, B. Dikmen, O. Canbolat, M. Kavutcu and M. Kacmaz, 1996a. Halothane hepatotoxicity and hepatic free radical metabolism in guinea pigs; the effects of vitamin E. *Can. J. Anaesth.*, 43: 741-748.
- Durak, I., O. Canbolat, M. Kavutcu, H.S. Oztürk and Z. Yurtarlani, 1996b. Activities of total, cytoplasmic and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patient with lung cancer. *J. Clin. Lab. Anal.*, 10: 17-20.
- Durak, I., O. Kurtipek, H.S. Ozturk, M. Birey, T. Guven, M. Kavutcu, M. Kacmaz, B. Dikmen, M. Yel and O. Canbolat, 1997. Impaired antioxidant defense in guinea pig heart tissues treated with halothane. *Can. J. Anaesth.*, 44: 1014-1120.
- Durak, I., H.S. Ozturk, B. Dikmen, C. Guven, M.Y. Cimen, S. Buyukkocak, M. Kacmaz and A. Avci, 1999. Isoflurane impairs antioxidant defense system in guinea pig kidney. *Can. J. Anaesth.*, 46: 797-802.
- Durak, I., M. Kavutcu, M. Kaçmaz, A. Avci, E. Horasanli, B. Dikmen, M.Y. Cimen and H.S. Ozturk, 2001. Effects of isoflurane on nitric oxide metabolism and oxidant status of guinea pig myocardium. *Acta Anaesthesiol. Scand.*, 45: 119-122.
- Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130-7139.
- Hirai, Y., 1987. Effect of isoflurane anesthesia on systemic and hepatic circulation in the dog. *JAMA*, 99: 1377-1390.
- Ignarro, L.J., G.M. Buga, K.S. Wood, R.E. Byrns and G. Chaudhuri, 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA*, 84: 9265-9269.
- Kang, M.J., H.S. Ahn and S.S. Lee, 2005. Effects of polyunsaturated/saturated fatty acid ratio and antioxidant supplementation on hepatic TBARS and enzyme activities under the maintenance of dietary peroxidizability index value in young and adult rats. *Ann. Nutr. Metab.*, 49: 304-311.
- Kang, M.Y., M. Tsuchiya, L. Packer and M. Manabe, 1998. *In vitro* study on antioxidant potential of various drugs used in the perioperative period. *Acta Anaesthesiol. Scand.*, 42: 4-12.
- Kim, J.W., J.K. No, Y. Ikeno, B.P. Yu, J.S. Choi, T. Yokozawa and H.Y. Chung, 2002. Age-related changes in redox status of rat serum. *Arch. Gerontol. Geriatr.*, 34: 9-17.
- Kohjitani, A., T. Miyawaki, M. Funahashi, H. Higuchi, R. Matsuo and M. Shimada, 2003. Ketamine and midazolam differentially inhibit nonadrenergic noncholinergic lower esophageal sphincter relaxation in rabbits: Role of superoxide anion and nitric oxide synthase. *Anesthesiol.*, 98: 449-458.
- Lind, R.C., A.J. Gandolfi and P.D. Hall, 1989. The role of oxidative biotransformation of halothane in the guinea pig model of halothane-associated hepatotoxicity. *Anesthesiol.*, 70: 649-653.
- Lopez-Diazguerrero, N.E., A. Luna-Lopez, M.C. Gutierrez-Ruiz, A. Zentella and M. Konigsberg, 2005. Susceptibility of DNA to oxidative stressors in young and aging mice. *Life Sci.*, 77: 2840-2854.
- Lowry, O., N. Rosenbraugh, L. Farr and R. Randall, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 182: 265-275.
- Prabhu, K.S., P.V. Reddy, E.C. Jones, A.D. Liken and C. Reddy, 2004. Characterization of a class alpha glutathione-S-transferase with glutathione peroxidase activity in human liver microsomes. *Arch. Biochem. Biophys.*, 424: 72-80.
- Richard, M.J., B. Portal, J. Meo, C. Coudray, A. Hadjian and A.E. Favier, 1992. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. *Clin. Chem.*, 38: 704-709.
- Runzer, T.D., D.M. Ansley, D.V. Godin and G.K. Chambers, 2002. Tissue antioxidant capacity during anesthesia: Propofol enhances *in vivo* red cell and tissue antioxidant capacity in a rat model. *Anesth. Analg.*, 94: 89-93.
- Schlack, W., B. Preckel, D. Stunneck and V. Thamer, 1998. Effects of halothane, enflurane, isoflurane, sevoflurane and desflurane on myocardial reperfusion injury in the isolated rat heart. *Br. J. Anaesth.*, 81: 913-919.
- Schmidt, C.C., S.W. Suttner, S.N. Piper, D. Nagel and J. Boldt, 1999. Comparison of the effects of desflurane and isoflurane anaesthesia on hepatocellular function assessed by alpha glutathione S-transferase. *Anaesthesia*, 54: 1204-1219.
- Schneemilch, C.E., T. Hachenberg, S. Ansorge, A. Ittenson and U. Bank, 2005. Effects of different anaesthetic agents on immune cell function *in vitro*. *Eur. J. Anaesthesiol.*, 22: 616-623.

- Sessa, W.C., 1994. The nitric oxide synthase family of proteins. *J. Vasc. Res.*, 31: 131-143.
- Sies, H., 1997. Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.*, 82: 291-295.
- Sullivan, J.C., E.D. Loomis, M. Collins, J.D. Imig, E.W. Inscho and J.S. Pollock, 2004. Age-related alterations in NOS and oxidative stress in mesenteric arteries from male and female rats. *J. Applied. Physiol.*, 97: 1268-1274.
- Tan, S., Y. Sagara, Y. Liu, P. Maher and D. Schubert, 1998. The regulation of reactive oxygen species production during programmed cell death. *J. Cell. Biol.*, 141: 1423-1432.
- Turkan, H., A. Aydin and A. Sayal, 2005. Effect of volatile anesthetics on oxidative stress due to occupational exposure. *World J. Surg.*, 29: 540-542.
- Xu, J., Y. Chang, B. Ouyang, Z. Lu and L. Li, 1998. Influence of isoflurane and sevoflurane on metabolism of oxygen free radicals in cardiac valve replacement. *Hunan Yi Ke Da Xue Xue Bao*, 23: 489-491.
- Van Ye, T.M., A.M. Roza, G.M. Pieper, J. Henderson, C.P. Johnson and M.B. Adams, 1993. Inhibition of intestinal lipid peroxidation does not minimize morphological damage. *J. Surg. Res.*, 55: 553-558.
- Wilson, J.X. and A.W. Gelb, 2002. Free radicals, antioxidants and neurologic injury: Possible relationship to cerebral protection by anesthetics. *J. Neurosurg. Anesthesiol.*, 14: 66-79.
- Wolin, M.S., M. Ahmad and S.A. Gupte, 2005. The sources of oxidative stress in the vessel wall. *Kidney Int.*, 67: 1659-1661.