# Laboratory Investigation

# Isoflurane impairs antioxidant defence system in guinea pig kidney

Ilker Durak PhD,* H. Serdar Öztürk MD,*
Bayazit Dikmen MD,‡ Cengiz Güven MD,†
M.Y. Burak Çimen MD,* Serap Büyükkoçak MD,*
Murat Kaçmaz MD,* Aslihan Avci MD*

**Purpose:** To investigate whether free radical metabolism is changed due to isoflurane treatment and, if so, to elucidate the role of changed free radical metabolism in the nephrotoxicity.

**Materials and methods:** Fifteen guinea pigs were used in the study. Five were treated with isoflurane in oxygen, five with oxygen and five were controls. Animals were exposed to isoflurane and oxygen three times. Each treatment was performed for 30 min once a day for three consecutive days. Activities of free radical enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px); values of antioxidant parameters, antioxidant potential (AOP), non-enzymatic superoxide radical scavenger activity (NSSA) and oxidation resistance (OR) and, level of an oxidant parameter namely, malondialdehyde (MDA) were determined in the renal tissues of the groups. Blood was also obtained for serum creatinine and urea analyses.

**Results:** AOP, NSSA, SOD and CAT activities were decreased; (0.0188 ± 0.0026 *vs* 0.0156 ± 0.0015, $P < 0.025$; 8.72 ± 1.80 *vs* 6.40 ± 1.22, $P < 0.05$; 76.71 ± 18.54 *vs* 52.79 ± 11.68, $P < 0.025$; 71.26 ± 15.58 *vs* 55.39 ± 8.83; $P < 0.05$, respectively) but, MDA level, OR value and GSH-Px activities increased (10.89 ± 1.57 *vs* 15.87 ± 2.97, $P < 0.01$; 0.84 ± 0.34 *vs* 2.28 ± 1.39, $P < 0.05$; 1.45 ± 0.83 *vs* 3.45 ± 1.20, $P < 0.01$, respectively) in kidney tissues from isoflurane-treated group compared with controls. No differences were observed between control and oxygen groups with regard to all analysis parameters except GSH-Px.

**Conclusion:** Isoflurane impairs the antioxidant defence system and this oxidant stress may play a part in the isoflurane-induced renal toxicity.

**Objectif :** Vérifier si le métabolisme des radicaux libres est changé par l'usage d'isoflurane et, si c'est le cas, préciser le rôle de ce métabolisme transformé sur la néphrotoxicité.

**Méthode :** L'étude a porté sur quinze cobayes dont cinq ont reçu de l'isoflurane dans de l'oxygène, cinq, de l'oxygène et cinq ont servi de témoins. Les animaux ont été exposés trois fois à l'isoflurane et à l'oxygène. Chaque traitement a été réalisé pendant 30 min, une fois par jour, trois jours consécutifs. On a déterminé dans les tissus rénaux des cobayes : les activités des enzymes des radicaux libres, la superoxyde-dismutase (SOD), la catalase (CAT) et la glutathion-peroxydase (GSH-Px); les valeurs des paramètres antioxydants, le potentiel antioxydant (PAO), l'activité non enzymatique des piégeurs de radicaux superoxydes (ANPS) et la résistance à l'oxydation (RO) ainsi que le niveau d'un paramètre oxydant, à savoir, le malondialdéhyde (MDA). On a aussi prélevé du sang aux fins d'analyses de la créatinine et de l'urée sériques.

**Résultats :** Les activités des PAO, ANPS, SOD et CAT étaient diminuées (0,0188 ± 0,0026 *vs* 0,0156 ± 0,0015, $P < 0,025$; 8,72 ± 1,80 *vs* 6,40 ± 1,22, $P < 0,05$; 76,71 ± 18,54*vs* 52,79 ± 11,68, $P < 0,025$; 71,26 ± 15,58 *vs* 55,39 ± 8,83; $P < 0,05$, respectivement) mais le niveau de MDA, la valeur de la RO et les activités de la GSH- Px augmentés (10,89 ± 1,57*vs* 15,87 ± 2,97, $P < 0,01$; 0,84 ± 0,34 *vs* 2,28 ± 1,39, $P < 0,05$; 1,45 ± 0,83 *vs* 3,45 ± 1,20, $P < 0,01$ respectivement) dans les tissus rénaux du groupe traité à l'isoflurane comparé au groupe témoin. Aucune différence n'a été relevée entre le groupe témoin et celui qui a reçu de l'oxygène quant aux analyses de tous les paramètres, sauf la GSH-Px.

**Conclusion :** L'isoflurane nuit au système de défense antioxydant et ce stress oxydant peut faire partie de la toxicité rénale induite par l'isoflurane.

From the Department of Biochemistry* and Department of Histology,† Medical Faculty, Ankara University and Dr.M. Ulker Emergency and Travmatology Hospital,‡ Anesthesiology Clinics, Turkey.

*Address correspondence to*: Prof. Dr. Ilker Durak, Ankara Universitesi Tip Fakültesi, Biyokimya Anabilim Dali (Dekanlik Binasi), 06100 Sihhiye, Ankara-Turkey. Phone: +90-312-3092219; Fax: +90-312-3106370; E-mail: durak@pallas.dialup.ankara.edu.tr

*Accepted for publication May 9, 1999*

ALL currently used volatile anesthetics are, to some extent, potentially toxic.[1] Hepatotoxicity and nephrotoxicity are important in this regard, because hepatocytes and proximal tubular cells are the two principal sites of anesthetic-induced toxic reactions.[2] Since some anesthetic agents including isoflurane, sevoflurane and desflurane contain fluoride in their chemical composition, it has been suggested that increased blood fluoride concentrations after anesthesia can be critical to the expression of fluorinated anesthetic-induced nephrotoxicity.[2,3] However, the mechanism by which fluoride causes proximal tubular necrosis has been poorly elucidated. In fact, some researchers found no evidence of nephrotoxicity despite high serum fluoride concentrations.[1,4] This supports the concept that serum fluoride concentration alone does not predict nephrotoxicity.[5]

Another potential mechanism, by which fluorinated anesthetics might contribute to the development of acute renal failure (ARF) is by a decrease in mean arterial blood pressure of 20-30 mm Hg after anesthesia.[6] This decrease in arterial blood pressure may produce renal hypoperfusion and ischemic tubular damage, which can increase the risk of developing nephrotoxic ARF.[7,8] As the perioperative period is frequently complicated by hypotension and nephrotoxic drug administration, fluorinated anesthetics may represent an additional risk factor for the development of ARF.[9,10]

In spite of these explanations, no molecular mechanism leading to fluorinated anesthetic-induced ARF has been documented in detail. In this study, isoflurane was selected for investigation because it exerts the greatest toxicity to the tubular cells and is the most widely used among fluorinated volatile anesthetics. The present study was undertaken to assess whether free radical-mediated cellular reactions played any part in anesthetic-induced nephrotoxicity.

## Materials and Methods

Fifteen guinea pigs (two months old, approx. 450 g) were used for the experiments. The study was performed according to the rules of Animal Care Committee of Health Ministry. There were three groups of five animals: control, isoflurane and oxygen. The animals were fed a laboratory diet during the study. Isoflurane, 2.0% v/v, and oxygen, 100%, mixture was given to the animals at 2 $L \cdot min^{-1}$ for 30 min a day for three consecutive days. In the oxygen group, only oxygen, 100%, was given under the same conditions. The gas mixture was inspired using face masks. At the end of the experiments, two hours after the last isoflurane treatment, the animals were killed and their kidneys were removed and placed in an ice bath until homogenisation for about one hour. Inferior vena cava blood was also withdrawn from the animals and used for measuring serum BUN and creatinine concentrations.

*Histological examination*: Part of each kidney was taken for light microscope examination. After fixation in formalin 10%, tissue samples were embedded in paraffin, cut at about 5 μm and stained with hematoxylen-eosin. The slides were coded and examined for morphological changes by light microscope.

*Biochemical analysis*: Kidneys were first washed with deionized water to remove blood and then homogenized in an homogenisator (B.Braun Melsungen model) at 1000 U for about three minutes. During tissue preparation procedure, the temperature was 4°C. After centrifugation, 10,000 g for about 60 min, the upper layer was taken. In this fraction SOD, glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured as described previously[11–13]. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the nitroblue tetrazolium salt (NBT) reduction rate. The CAT and GSH-Px activities were given in $IU \cdot mg^{-1}$ protein and SOD activity in $U \cdot mg^{-1}$ protein. The malondialdehyde (MDA) concentration was determined by using the thiobarbituric acid reaction[14] and the amount of protein by the Lowry method.[15] The anti-oxidant potential (AOP), oxidation resistance (OR) and non-enzymatic superoxide radical scavenger activity (NSSA) analyses were performed as described previously.[16–18] Serum urea and creatinine concentrations were measured in the routine biochemistry laboratory.

The AOP was assessed as described below, which is based on the determination of MDA levels before and after exposure to superoxide radicals produced by xanthine-xanthine oxidase system.[16] In the reaction medium enriched with fish oil, samples (supernatant obtained after centrifugation) were exposed to superoxide radicals ($O_2^{\bullet -}$) produced by xanthine/xanthine oxidase system for one hour. We think that, by using this reaction system, it is possible to obtain more correct information of the total (enzymatic and nonenzymatic) antioxidant potential (AOP) of the tissue and cells. For this, MDA levels of the reaction medium ($nmol \cdot g^{-1}$ tissue) were measured before (blank) and after (sample) $O_2^{\bullet -}$ radical attack. The difference between both values was inversely proportional with AOP of the tissues ($1 \cdot nmol^{-1} \cdot g\ tissue^{-1} hr^{-1}$). Antioxidant potential was assessed from the difference between MDA levels of blank and sample studies and results were expressed as $1 \cdot nmol \cdot g\ tissue^{-1} hr^{-1}$.

The OR was assessed as described below, which mainly based on the determination of MDA levels

TABLE I Sensitivity (detection limit) and variability (% CV) of the parameters measured in the study.

| | *MDA* | *AOP* | *OR* | *CAT* | *GSH-Px* | *SOD* | *NSSA* |
|---|---|---|---|---|---|---|---|
| Detection limit | 0.80 $nmol \cdot g^{-1}$ | 0.0155 $nmol^{-1} \cdot g^{-1} \cdot h^{-1}$ | 0.0044 $nmol^{-1} \cdot g^{-1} \cdot h^{-1}$ | 3.50 $IU \cdot mg^{-1}$ | 0.005 $IU \cdot mg^{-1}$ | 0.10 $U \cdot mg^{-1}$ | 0.10 U·mg |
| % CV (within) | 7.8 | 4.2 | 7.9 | 3.7 | 4.2 | 2.1 | 2.3 |
| % CV (between) | 8.3 | 5.1 | 9.0 | 4.5 | 4.8 | 2.5 | 2.6 |

TABLE II Mean ± SD values of MDA ($nmol \cdot g^{-1}$), AOP ($nmol^{-1} \cdot g^{-1} \cdot hr^{-1}$), OR ($nmol^{-1} \cdot g^{-1} \cdot hr^{-1}$), CAT ($IU \cdot mg^{-1}$), GSH-Px ($mIU \cdot mg^{-1}$), SOD ($U \cdot mg^{-1}$) and NSSA ($U \cdot mg^{-1}$) in the kidney tissues of the groups and, serum Creatinine ($mg \cdot dl^{-1}$) and BUN ($mg \cdot dl^{-1}$) levels.

| *Groups* | *MDA* | *AOP* | *OR* | *CAT* | *GSH-Px* | *SOD* | *NSSA* | *Creatinine* | *BUN* |
|---|---|---|---|---|---|---|---|---|---|
| I (n=5) | 10.89 ± 1.57 | 0.094 ± 0.013 | 0.84 ± 0.34 | 71.26 ± 15.58 | 1.45 ± 0.83 | 76.71 ± 18.54 | 8.72 ± 1.80 | 0.45 ± 0.05 | 30.75 ± 2.48 |
| II (n=5) | 15.87 ± 2.97 | 0.078 ± 0.007 | 2.28 ± 1.39 | 55.39 ± 8.83 | 3.45 ± 1.20 | 52.79 ± 11.68 | 6.40 ± 1.22 | 0.48 ± 0.07 | 22.80 ± 3.37 |
| III (n=5) | 12.20 ± 4.20 | 0.101 ± 0.006 | 1.35 ± 0.75 | 81.01 ± 7.33 | 4.45 ± 1.38 | 65.85 ± 6.54 | 8.12 ± 2.02 | 0.40 ± 0.05 | 22.25 ± 4.91 |
| ANOVA | F=3.89 | F=8.27 | F=3.06 | F=6.70 | F=8.68 | F=4.11 | F=2.50 | - | - |
| | $P < 0.05$ | $P < 0.01$ | $P < 0.1$ | $P < 0.025$ | $P < 0.005$ | $P < 0.05$ | $P < 0.25$ | | |
| Mann Whitney U test | | | | | | | | | |
| I-II | $P < 0.01$ | $P < 0.025$ | $P < 0.05$ | $P < 0.05$ | $P < 0.01$ | $P < 0.025$ | $P < 0.05$ | n.s. | n.s. |
| I-III | n.s. | n.s. | n.s. | n.s. | $P < 0.005$ | n.s. | n.s. | n.s. | n.s. |
| II-III | n.s. | $P < 0.0005$ | n.s. | $P < 0.005$ | n.s. | $P < 0.05$ | $P < 0.025$ | n.s. | n.s. |

I-Control, II-Isoflurane, III-Oxygen
n.s.: non-significant ($P > 0.05$)

before and after incubation with copper II sulphate. By incubation, metal-catalysed peroxidation is induced and then peroxidation products (malondialdehyde by thiobarbituric acid assay) are determined. By this reaction system, it is possible to obtain data into the oxidation status of tissue and plasma samples; if the sample under investigation was previously exposed to oxidant stress and could not cope with it, then OR value would be increased.[17]

The (NSSA) measurement was assessed to obtain correct superoxide dismutase (SOD) enzyme activity. In the present SOD measurement methods, total (enzymatic plus non-enzymatic) superoxide scavenger activity instead of SOD activity was measured. To obtain enzymatic (SOD) and non-enzymatic superoxide scavenger activities (NSSA) separately, samples were exposed to trichloroacetic acid (TCA) treatment. Thus, SOD enzyme protein together with other proteins were removed by precipitation.[18]

The precision analysis results (within, 10 analyses with the same sample on the same day, and between, one analysis with the same sample on consecutive 10 days, batch analyses) and, the sensitivity limits of the parameters measured in the study are given in Table I.

In the statistical analysis, ANOVA and Mann-Whitney U tests were used.

## Results

The AOP, NSSA, SOD and CAT activities were decreased but, MDA concentration, OR value and GSH-Px activities increased in isoflurane-treated group compared with controls, indicating impaired antioxidant defence in the kidney tissues (Table II). No differences were observed between control and oxygen groups with regard to all analysis parameters except GSH-Px. There were no differences between serum urea and creatinine concentrations among the groups, indicating absence of functional renal impairment.

Histopathological examination revealed glomerular congestion, glomerular hypercellularity, necrosis in proximal tubular cells and cellular residues in distal tube lumens in isoflurane group (Figures 1, 2, 3). In the oxygen group, minimal glomerular congestion and loss of cytoplasm in some proximal tubular cells were observed (Figure 4). There was no histopathological abnormality in the control samples (Figure 5).

## Discussion

The results of the present study show that isoflurane treatment impairs enzymatic and non-enzymatic antioxidant defence systems in renal tissue. Isoflurane and/or one of its metabolites may play a part in the inhibition of SOD and CAT enzymes. For example,

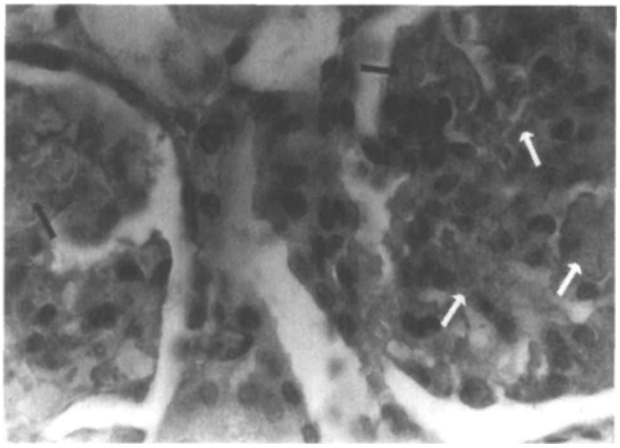

FIGURE 1 Photomicrograph of renal tissue from isoflurane-treated guinea pigs.
‣ glomerular congestion. H.E. × 250

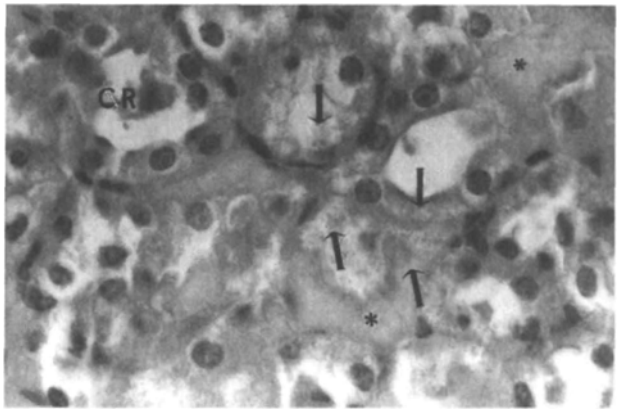


FIGURE 3 Photomicrograph of renal tissue from isoflurane-treated guinea pigs. loss of cytoplasm, *homogenization, ‣ caryolysis, CR: cellular residue in distal tube lumen. H.E. × 250

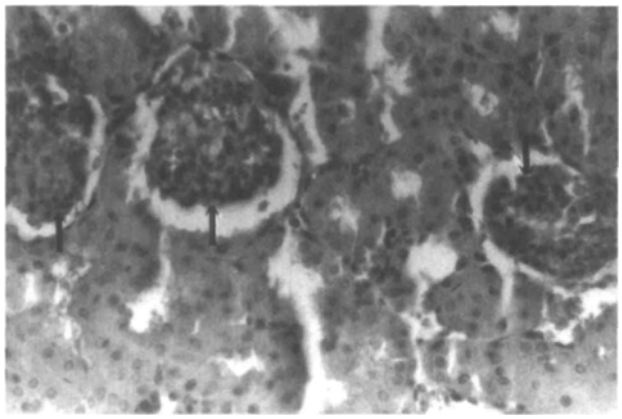

FIGURE 2 Photomicrograph of renal tissue from isoflurane-treated guinea pigs.
→ glomerular hypercellularity. H.E. × 100

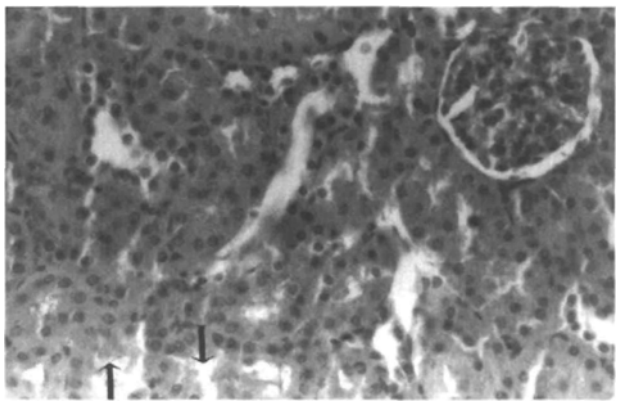

FIGURE 4 Photomicrograph of renal tissue from oxygen-treated guinea pigs. Minimal congestion in glomerulus, loss of cytoplasm. H.E. × 100

fluoride which is a constituent of isoflurane and the most electronegative element[21] may inhibit these enzymes by making complexes with their cofactor metals such as $Cu^{++}$, $Zn^{++}$ and $Fe^{++}$. However, GSH-Px activity was found to be higher in the isoflurane group than in the control group and lower than in the oxygen group. This demonstrates that increased renal GSH-Px activity in the isoflurane group is mainly due to oxygen exposure rather than isoflurane treatment. It is possible that, although isoflurane makes inhibition in GSH-Px activity, this effect is masked by the inductive effect of oxygen (Table II). In addition to total antioxidant defence potential (AOP), non-enzymatic antioxidant defence (NSSA) is also weakened, possibly because the oxidant stress created by isoflurane treatment leads to depletion of non-enzymatic antioxidant system as well. Increased oxidation resistance also supports the hypothesis that isoflurane treatment causes preoxidation in cellular structures. It seems that isoflurane treatment results in considerable impairment of the antioxidant defence system and leads to oxidant stress in the renal tissue, the indication for which is increased MDA levels in the affected tissue. Because of the reduced antioxidant defence

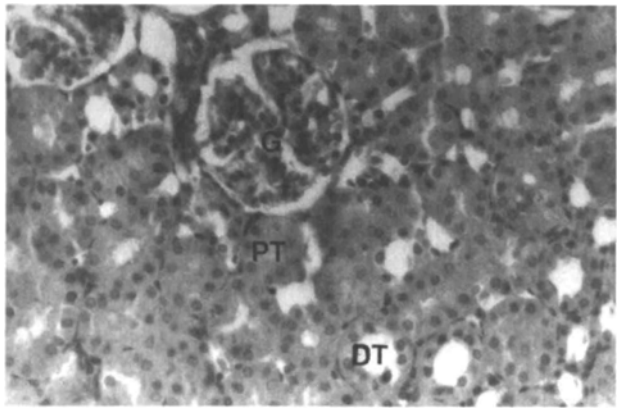


FIGURE 5 Photomicrograph of renal tissue from control group. G: glomerulus, DT: distal tubulus, PT: proximal tubulus. H.E. x 100

capacity (decreased AOP and NSSA values), tubular cells are exposed to free radical-mediated peroxidation reactions. Since the cellular structures are undergone to pre-oxidation, they gain resistance against $Cu^{++}$-induced oxidation. Accordingly, histopathological examination results reveal considerable derangements such as glomerular congestion, necrosis in proximal tubular cells and cellular residues in distal tubular lumens in the affected tissue (Figures 1, 2, 3). Despite these changes, no functional insufficiency was observed in the isoflurane-treated kidney tissues (unchanged BUN and creatinine levels).

Toxicity mechanisms, by which inhaled flourinated anesthetics contribute to renal damage have been investigated for some time but no satisfactory explanation have been obtained.[19] Some investigators suggested that the nephrotoxicity of fluorinated volatile compounds arises from inorganic fluoride,[20] which may inhibit enzyme systems by binding tightly to metal cations.[21] However, no evidence of nephrotoxicity has been found despite high serum fluoride concentrations[1,4] so that fluoride concentrations alone did not predict anesthetic nephrotoxicity.[5] Anesthesia-associated hypotension and resulting tissue ischemia was also suggested as one of the factors leading to tubular nephrotoxicity.[7,8] However, no satisfactory evidence of these molecular mechanisms have been obtained. In our opinion, increased fluoride concentrations due to anesthetic treatment might impair antioxidant defence system by making powerful metal complexes.

In conclusion, our results suggest that impaired enzymatic and nonenzymatic antioxidant defence systems due to isoflurane treatment leads to oxidant stress in the tubular cells and accelerates peroxidation reactions in the renal tissue. Antioxidant treatment may give beneficial results in this regard but requires further verification.

## References

1 *Kharasch ED, Frink EJ Jr, Zager R, Bowdle TA, Artru A, Nogami WM.* Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity. Anesthesiology 1997; 86: 1238–53.

2 *Whitford GM.* The Metabolism and Toxicity of Fluoride. Monographs in Oral Science, Vol 13. Basel: Karger, 1989: 1–160.

3 *Lochhead KM, Kharasch ED, Zager RA.* Spectrum and subcellular determinants of fluorinated anesthetic-mediated proximal tubular injury. Am J Pathol 1997; 150: 2209–21.

4 *Kharasch ED.* Sevoflurane and the kidney: a current perspective. Ann Anesthetic Pharmacol 1997; 1: 205–22.

5 *Kharasch ED, Hankins DC, Thummel KE.* Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. Anesthesiology 1995; 82: 689–99.

6 *Marsnail BE, Longnecker DE.* General anesthetics. *In*: Goodman and Gilman's The Pharmacologic Basis of Therapeutics, 9th ed. New York: Mc Graw-Hill, 1996: 307–30.

7 *Zager RA.* Gentamicin nephrotoxicity in the setting of acute renal hypoperfusion. Am J Physiol 1988; 254: F574–81.

8 *Zager RA.* Myoglobin depletes renal adenine nucleotide pools in the presence and absence of shock. Kidney Int 1991; 39: 111–9.

9 *Kellerman PS.* Perioperative care of the renal patient. Arch Int Med 1994; 154: 1674–88.

10 *Novis BK, Roizen MF, Aronson S, Thisted RA.* Association of preoperative risk factors with postoperative acute renal failure. Anesth Analg 1994; 78: 143–9.

11 *Durak I, Canbolat O, Kavutcu M, Öztürk HS, Yurtarslaný Z.* Activities of total, cytoplasmic and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal 1996; 10: 17–20.

12 *Paglia DE, Valentine WN.* Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158–69.

13 *Aebi H.* Catalase. *In*: Bergmeyer HU (Ed.). Methods of Enzymatic Analysis. New York: Academic Press, 1974: 673–7.

14 *Dahle LK, Hill EG, Holman RT.* The thiobarbituric acid reaction and the autoxidations of polyunsaturated

fatty acid methyl esters. Arch Biochem Biophys 1962; 98: 253–61.

15 *Lowry O, Rosenbraugh N, Farr L, Randall R.* Protein measurement with folin phenol reagent. J Biol Chem 1951; 182: 265–75.

16 *Durak I, Karabacak HI, Büyükkoçak S et al.* Impaired antioxidant defense system in the kidney tissues from rabbits treated with cyclosporine. Protective effects of vitamins E and C. Nephron 1998; 78: 207–11.

17 *Dasgupta A, Zdunek T.* In vitro lipid peroxidation of human serum catalysed by cupric ion: antioxidant rather than prooxidant role of ascorbate. Life Sci 1992; 50: 875–82.

18 *Durak I, Canbolat O, Kaçmaz M, Özgen G, Öztürk HS.* Antioxidant interferences in superoxide dismutase activity methods using superoxide radical as substrate. Clin Chem Lab Med 1998; 36: 407–8.

19 *Kenna JG, Jones RM.* The organ toxicity of inhaled anesthetics. Anesth Analg 1995; 81: S51–66.

20 *Mazze RI, Cousins MJ, Kosek J.* Dose-related methoxyflurane nephrotoxicity in rats: a biochemical and pathologic correlation. Anesthesiology 1972; 36: 571–87.

21 *Ellenhorn MJ, Barceloux DG.* Medical Toxicology. New York: Elsevier, 1988.