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Effects of Halothane Anesthesia in Unilateral Nephrectomized Dog: Histopathological and Biochemical Findings

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Abstract: The aim of this study is evaluation of the effects of halothane anesthesia following unilateral nephrectomized dogs and study of biochemical and histopathological effects. Six healthy 10-12 months old dogs weighing 25.7 ± 2.5 kg were used in this study. Left unilateral nephrectomy was performed in all dogs. After nephrectomy the experiment was performed in two stages: in first stage dogs for 3 h and second stage dogs for 6 h were anesthetized using halothane in oxygen. Arterial blood samples and venous blood samples were collected for blood gas analysis and for measurement of BUN, Creatinin and Serum Fluoride Concentration (SFC) during the study. Following the first halothane anesthesia, BUN and Creatinin were significantly increased from 24 to 48 h compared to pre-nephrectomy, post-nephrectomy and pre-anesthesia in the second stage of the experiment, the significantly increases in BUN and Creatinin in comparison to pre-nephrectomy, post-nephrectomy and pre-anesthesia from 24 to 72 h and significantly increase in SFC concentration observed 3 to 72 h after anesthesia in comparison to pre-nephrectomy, post-nephrectomy and pre-anesthesia.

Key words: Unilateral nephrectomy, halothane, dog

INTRODUCTION

Inhalant anesthetics are used widely for producing general anaesthesia in animals and human. Although largely removed from the body via the lungs, the inhalant anesthetics are not chemically inert and undergo varying degrees of metabolism, approximately 20% of halothane uptake is metabolized via oxidative and reductive pathways during and following halothane anaesthesia in humans (Reichle and Conzen, 2003). The major metabolite of oxidative pathway, trifluoroacetic acid, is a stable end product, which is eliminated in the urine (Sharifi and Vesal, 2005); Trifluoroethane, difluoroethylene and fluoride ion are major metabolites of the reductive pathway (Spracklin *et al.*, 1996).

Inorganic fluoride is a common metabolite of halogenated inhalation anesthetics. The degradation of methoxyflurane (Kharasch *et al.*, 1995) halothane enflurane isoflurane (Kharasch *et al.*, 2001) and sevoflurane (Bouche, 1991) has been associated with transient postanesthetic increases in serum fluoride levels in mice, rats, guinea pigs, dogs and humans. Since trifluoroethane and difluoroethylene are volatile and eliminated by expiration, serum fluoride is a more reliable indicator of the degree of reductive metabolism of halothane. The extent of metabolism of inhalant anesthetics depends on a variety of factors including hepatic enzyme activity (Elliott and Strunin, 2003), the blood concentration of anesthetic (Sharifi and Vesal, 2005), genetic factors and sex (Sharifi and Vesal, 2005).

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Kharasch *et al.* (2001) showed renal deficiency following halothane and methoxyflurane anesthesia resulted from increase of fluoride ion and introduced BUN and Creatinin as gold standards for evaluation of the condition.

Wickstroem and Stefansson (1981) reported that significant difference between BUN and Creatinin can be a result of repeated halothane anesthesia in long duration that results in renal injuries and finally reduced glomerular filtration rate and increase of BUN and Creatinin. Significant increase of BUN and Creatinin in addition to increase of renal output was reported in dogs following 6 h halothane anesthesia.

Toxic effects of halothane on liver, kidney and brain were studied. Liver degeneration is a finding after halothane anesthesia in different species. Recent electron microscopy studies also showed cell degeneration in liver cells after clinical halothane anesthesia. Kidneys and nervous system degeneration also were reported after halothane anesthesia. Pathologic effects of halothane on fetal liver, kidney and brain also were reported by electron microscopic studies. However correlation of these findings with humane is not completely obvious but potential toxicity of the halothane on biological systems is evident and needs more studies (Bouche, 1991).

In another study by Higuchi *et al.* (1998) they found renal injury as defined by increased urinary levels of N-acetyl-B-glucosaminidase correlated with increased inorganic fluoride levels produced by sevoflurane biodegradation.

Mazze *et al.* (2000) have been showed nephrotoxic effects of halothane anesthesia in patient with kidney deficiencies.

In order to study the effects of halothane anesthesia we have used unilateral nephrectomized dogs as an animal model.

MATERIALS AND METHODS

This study was conducted at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Shahrekord University in September 2007. The Institutional Animal Care and Use Committee approved the protocol for this project. Six healthy 10-12 months old dogs weighing 25.7 ± 2.5 kg (mean $\pm$ SD) were used in this study. The dogs were confined to indoor kennel and were vaccinated for common infectious diseases and physical examination was performed for the health status of each subject. All animals were allowed an acclimation period of two weeks prior to the beginning of the study. Before each anesthetic procedure, a complete blood cell count and serum chemical profile indicated no abnormalities.

Left unilateral nephrectomy was performed in all dogs. After nephrectomy the experiment was performed in two stages: in first stage dogs were anesthetized for 3 h and in second stage for 6 h, respectively using halothane in oxygen. The animals were fasted for 18 h before the induction of anesthesia. No sedatives or tranquilizers were administered to the dogs. Prior to anesthesia, the left cephalic vein was catheterized for subsequent fluid administration and venous blood sampling. For induction of anesthesia, dogs were gently restrained and halothane in 100% oxygen (4 L min^{-1}) was delivered via a fitted facemask. The concentration of halothane was gradually increased (0.5% every 30 sec) until a vaporizer setting of 4% was reached. Following intubation, dogs were connected to a rebreathing system and a medium plane of anesthesia, as determined by palpebral and pedal reflexes, was maintained with halothane (1-1.2%) in oxygen (1.5 L min^{-1}). After induction of anesthesia, a 22-gauge catheter was placed percutaneously into the femoral artery for collection of arterial blood samples. Lactated Ringer's solution (10 mL/kg/h) was administered during anesthesia. Ventilation was supported with intermittent positive pressure ventilation (tidal volume 15 mL kg^{-1} , respiratory rate 10-12 breaths/min). Arterial blood samples were collected anaerobically at 30, 60 and 120 min after

induction and immediately analyzed using a blood gas analyzer. The blood gas values were corrected for body temperature and hemoglobin concentration. Venous blood samples were obtained prior to induction of anesthesia (time 0) and 3, 6, 24, 48 and 72 h following initiation of anesthesia for measurement of BUN and Creatinin. Serum Fluoride Concentration (SFC) was measured by an ion-specific potentiometric method 20. Heart rate was obtained by use of a continuous ECG monitoring rectal temperature was measured at one-hour intervals. The dogs were placed in right lateral recumbency on a padded surgical table during maintenance of anesthesia. At the end of anesthesia, the dogs were disconnected from the anesthetic circuit and were allowed to breathe room air. Induction time (time from administration of halothane to tracheal intubation), extubation time (time from discontinuation of halothane to swallowing reflex) and time to sternal recumbency (time from discontinuation of halothane to sternal recumbency) were also recorded during anesthetic induction and recovery.

Data Analysis

All data are presented as mean±SD, unless otherwise stated. A one-way ANOVA followed by Duncan test was used to compare postanesthesia SFCs, BUN and Creatinin concentration with preanesthesia, postnephrectomy and pre-nephrectomy values.

RESULTS

It was relatively easy to unilateral nephrectomy in dogs. Unilateral nephrectomy was performed without difficulties and no complications were encountered during surgery or postoperatively. Mask induction was easily performed in dogs with minimal restraint. Induction of anesthesia was smooth and no struggling or objection to placement of the mask was observed.

The measurement of BUN and Creatinin showed no significant differences between pre-nephrectomized and two week later post-nephrectomized dogs. Following the first halothane anesthesia, SFCs weren't significantly increased from 3 to 72 h post-anesthesia compared to pre-anesthesia, pre-nephrectomy and postnephrectomy ($p = 0.05$, Table 1) but significant difference was recorded in BUN from 24 to 48 h and Creatinin from 24 to 48 postanesthesia compared to preanesthesia, pre-nephrectomy and postnephrectomy. Significant increase was recorded in BUN and Creatinin, after anesthesia in comparison with pre-nephrectomy, post-nephrectomy and preanesthesia in second stage from 24 to 72 h and CSF concentration from 3 to 72 h after anesthesia ($p < 0.05$, Table 2).

Table 1: Serum fluoride concentration (ppm), BUN and Creatinin ($\mu\text{m dL}^{-1}$) during in the first stage of experiment

Blood bio-chemistry factors	Times of blood sampling		First part of examination					
	Before nephrectomy	After nephrectomy	Before anesthesia	3 h After anesthesia	6 h after anesthesia	24 h after anesthesia	48 h after anesthesia	72 h after anesthesia
BUN	7.360±34.167	5.574±37.333	2.280±39	2.639±43.833	5.241±44.333	4.274±45.667*	8.359±56.333*	5.538±57.312
Creatinin	0.124±0.667	0.110±0.830	0.165±0.82	0.268±8927.0	0.225±0.888	0.267±0.965*	0.286±1.082*	0.218±1.027
Fluoride	0.0319±0.122	0.0187±0.125	0.0147±0.142	0.0358±0.150	0.0366±0.168	0.0695±0.171	0.0729±0.170	0.219±0.172

Data are given as Mean±SD. *Significant difference of BUN pre-nephrectomy, postnephrectomy and preanesthesia from baseline ($p \leq 0.05$).

*Significant difference of Creatinin pre-nephrectomy, postnephrectomy and preanesthesia from baseline ($p \leq 0.05$).

Table 2: Serum fluoride concentration (ppm), BUN and Creatinin ($\mu\text{m dL}^{-1}$) during in the second stage of experiment

Blood bio-chemistry factors	Times of blood sampling		First part of examination					
	Before nephrectomy	After nephrectomy	Before anesthesia	3 h After anesthesia	6 h after anesthesia	24 h after anesthesia	48 h after anesthesia	72 h after anesthesia
BUN	7.360±34.167	5.574±37.333	3.971±43.833	5.203±46.353	5.391±48.323	9.274±94*	9.028±101.500*	6.976±122.667*
Creatinin	0.124±0.667	0.110±0.830	0.420±0.856	0.255±0.854	0.231±0.861	0.188±1.442*	0.217±1.780*	0.281±1.798*
fluoride	0.0319±0.122	0.0187±0.125	0.283±0.5	0.306±2.617*	0.264±2.583*	0.366±2.417*	0.366±2.417*	0.319±2.683*

Data are given as Mean±SD. *Significant difference of BUN pre-nephrectomy, postnephrectomy and preanesthesia from baseline ($p \leq 0.05$).

*Significant difference of Creatinin pre-nephrectomy, postnephrectomy and preanesthesia from baseline ($p \leq 0.05$). *Significant difference of fluoride pre-nephrectomy, postnephrectomy and preanesthesia from baseline ($p \leq 0.05$).

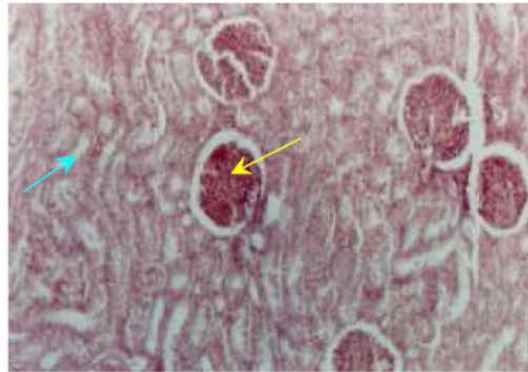


Fig. 1: Degeneration and vacuolation in epithelium tubular cells and glomerular hyperemia and hypercellularity

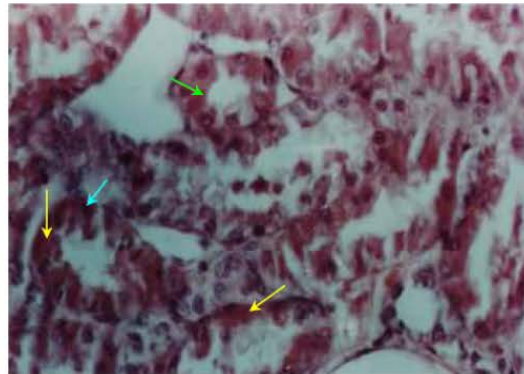


Fig. 2: Necrosis of some epithelial cells of the urinary tubules with nuclear piknosis

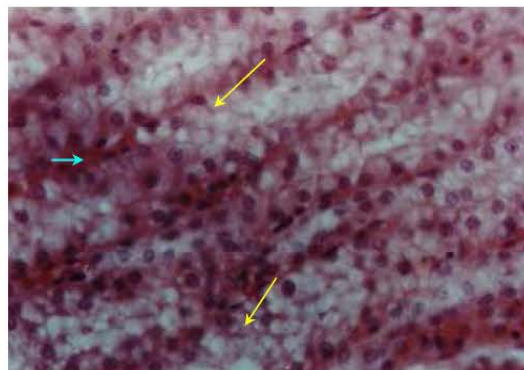


Fig. 3: Cell swelling and necrosis of some tubular epithelial cells

Histopathological results on remains kidney showed necrosis of the urinary tubules, hyperemia of glomerulus's and cell degeneration (Fig. 1-3).

No significant changes in rectal temperature and heart rate or rhythm were detected. All dogs recovered uneventfully from anesthesia; no adverse reactions or complications were encountered in this study.

DISCUSSION

The baseline SFC, BUN and Creatinin for this study is consistent with that has been reported for dogs (Stone *et al.*, 2002). In the present study, anesthesia was induced with halothane delivered by mask, so any possible effects of premeditations or injectable anesthetic agents on halothane metabolism could be eliminated. Although we did not measure the inhaled concentration of halothane, the vaporizer setting and the depth of anesthesia was the same in all dogs. Since no surgery was performed during anesthesia, it was easy to maintain a constant light level of anesthesia throughout the 3 and 6 h anesthesia period.

Most drugs metabolized via liver oxidation/reduction and in following conjugation excreted from gall or urine. Clinically, changes in metabolizing enzymes are important. Changes in effects of the drugs may lead to increasing or decreasing liver enzymes activity. For example use of barbiturates before halothane anesthesia may increase toxic metabolites of the halothane organofluoride that could leave toxic effects on kidney and liver (Thurmon *et al.*, 1996).

Methoxyflurane nephrotoxicity directly related to metabolism of the drug and production of non organic fluorides ions. Increase metabolism following enzymatic induction resulted in increase of non organic fluoride production. Enzymatic induction of phenobarbital and phenytoin resulted in increase fluorination of methoxyflurane but no effects were reported on enflurane and isoflurane in rats.

Methoxyflurane more detoxified in-vitro in comparison to other drugs. These studies show that induction of anesthesia with enzymatic induced medications in case of methoxyflurane anesthesia can increase nephrotoxicity (Burtis and Ashwood, 1994).

Increase of serum fluoride during and after halothane, sevoflurane and enflurane anesthesia in rat and human were reported by Levine *et al.* (1996).

Toxic effects of methoxyflurane in kidneys are decrease glomerular filtration rate with increase in blood urine nitrogen and keratinine. These effects following increase of fluoride Ion to 50-80 $\mu\text{mol L}^{-1}$ started and when it reaches to 80-175 $\mu\text{mol L}^{-1}$ renal toxicity is completely obvious (Cousins *et al.*, 1976).

Clinical studies with methoxyflurane showed that concentrations more than 50 $\mu\text{mol L}^{-1}$ of non organic fluoride Ion can be nephrotoxic. By the time no renal defects following enflurane and isoflurane were reported. Also renal toxicity of sevoflurane related to fluoride Ion not only in animals but also in humane following long term anesthesia in patients with the history of renal diseases weren't reported. 50 $\mu\text{mol L}^{-1}$ is the threshold of renal toxicity of fluoride Ion that is not the case in isoflurane, enflurane and sevoflurane anesthesia. It seems that fluoride Ion is increased following anesthesia with these drugs without any clinical importance (Mazze *et al.*, 2000).

Half-life of non organic fluoride is 90 min in serum but after methoxyflurane anesthesia high fluoride level will last till 3 days but after enflurane and isoflurane anesthesia it drops rapidly. Methoxyflurane is 10 times more soluble in lipid than enflurane and isoflurane and will last many days after anesthesia, so a source of non organic fluoride will be available after anesthesia (Sharifi and Vesal, 2005).

In this study significant elevation of BUN and serum Creatinin after anesthesia in comparison to before anesthesia and before and after nephrectomy following fluoride elevation in first stage anesthesia were proved. Also significant increase of these factors after significant increase of fluoride Ion after anesthesia in comparison to the above mentioned times can be a result of nephrotoxic action of fluoride on kidneys.

In the early case, reports of fulminant hepatic failure after halothane anesthesia, patients often had a history of recent previous exposure to halothane (Iuman and Mushin, 1974). Auto-antibodies to body tissues such as liver, kidney microsomes thyroid nucleic content smooth muscle and mitochondria and eosinophilia, rash and fever were sometimes present (Pumford *et al.*, 1993; Moulton and Sherlock, 2001). These features resemble an idiosyncratic drug reaction. As halothane is a small molecule unlikely to be immunoreactive (Brown and Gandolfi, 2001), it was postulated that binding of a metabolite of halothane to the liver cytochromes could act as a hapten and induce a hypersensitivity response (Utrecht, 1997).

Repeated halothane anesthesia may result in elevation of liver microsomal enzymes and finally increase halothane metabolism that result in non organic fluoride that leads to toxic effects on kidneys and elevation of the above mentioned factors (Topal *et al.*, 2003).

Studies showed that reduced oxygen before activation of liver enzymes or long term reduced arterial blood pressure may increase renal and liver damages following halothane anesthesia (Behnia *et al.*, 2003).

Creatinin is excreted by kidneys so it relates with glomerular filtration rate. Clinically this index can be used as a scale for glomerular filtration rate. Following unilateral renal disease or nephrectomy in case of normal function of remainder kidney reduction in glomerular filtration rate is anticipated (Mazze *et al.*, 2000) therefore Serum Creatinin were measured in second week after anesthesia in order to clarify normal function of right kidney, results were in normal range of Creatinin ($0.8-1.8 \text{ mg dL}^{-1}$).

Level of oxygen and carbon dioxide monitored by blood gas analysis and dogs didn't show any hypoxemia or carbon dioxide accumulation during anesthesia. Hypoxia may alter halothane metabolism (Ponte and Sadler, 1989). Regarding to design of study other factors like liver, renal and cardiopulmonary systems were the same for each animal and the only difference between two stages was duration of anesthesia. It seems that duration of anesthesia can increase halothane anesthesia and production of non organic fluoride Ion that result in increased nephrotoxicity.

REFERENCES

- Behnia, R., A. Molteni and R. Igic, 2003. Angiotensin-converting enzyme inhibitors: Mechanisms of action and implications in anesthesia practice. *Curr. Pharm. Des.*, 9: 763-776.
- Bouche, M., 1991. Absorption and degradation of sevoflurane and isoflurane in a conventional anesthetic circuit. *Anesth Analg.*, 72: 785-789.
- Brown, B.R. and A.J. Gandolfi, 2001. Adverse effects of volatile anaesthetics. *Br. J. Anaesth.*, 59: 14-23.
- Burtis, C.A. and E.R. Ashwood, 1994. *Tietz Textbook of Clinical Chemistry*. 2nd Edn., W.B. Saunders Co., Philadelphia, ISBN-10: 0721656102, pp: 735-888, 1354-1375.
- Cousins, M.J., L.R. Greenstein, B.A. Hitt and R.I. Mazze, 1976. Metabolism and renal effects of enflurane in man. *Anesthesiology*, 44: 44-53.
- Elliott, R.H. and L. Strunin, 2003. Hepatotoxicity of volatile anaesthetics. *Br. J. Anaesth.*, 70: 339-348.
- Higuchi, H., S. Sumita, H. Wada, T. Ura and T. Ikemoto *et al.*, 1998. Effects of sevoflurane and isoflurane on renal function and on possible markers of nephrotoxicity. *Anesthesiology*, 89: 307-322.
- Iuman, W. and W. Mushin, 1974. Jaundice after repeated exposure to halothane: An analysis of reports to the committee on safety of medicines. *Br. Med. J.*, 1: 5-10.
- Kharasch, E.D., D.C. Hankins and K.E. Thummel, 1995. Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. *Anesthesiology*, 82: 689-699.

- Kharasch, E.D., E.J. Frink, A. Artru, P. Michalowski and G.A. Rooke *et al.*, 2001. Long-duration low-flow sevoflurane and isoflurane effects on postoperative renal and hepatic function. *Anesth. Analg.*, 93: 1511-1520.
- Levine, M.F., J. Sarnar, J. Lerman, P. Davis and N. Sikich *et al.*, 1996. Plasma inorganic fluoride concentrations after sevoflurane anesthesia in children. *Clin. Invest. Anesthesiol.*, 84: 348-353.
- Mazze, R.I., C.M. Callan, S.T. Galvez, L. Delgado-Herrera and D.B. Mayer, 2000. The effects of sevoflurane on serum creatinine and blood urea nitrogen concentrations: A retrospective, twenty-two-center, comparative evaluation of renal function in adult surgical patients. *Anesth. Analg.*, 90: 683-688.
- Moult, P.J.A. and S. Sherlock, 2001. Halothane-related hepatitis a clinical study of twenty-six cases. *QJM.*, 44: 99-114.
- Ponte, J. and C.L. Sadler, 1989. Effect of halothane, enflurane and isoflurane on carotid body chemoreceptor activity in the rabbit and the cat. *Br. J. Anaesth.*, 62: 33-40.
- Pumford, N.R., B.M. Martin, D. Thomassen, J.A. Burris and J.G. Kenna *et al.*, 1993. Serum antibodies from halothane hepatitis patients react with the rat endoplasmic reticulum protein ERp72. *Chem. Res. Toxicol.*, 6: 609-615.
- Reichle, F.M. and P.F. Conzen, 2003. Halogenated inhalational anaesthetics. *Best Pract. Res. Clin. Anaesthesiol.*, 17: 29-46.
- Sharifi, S. and N. Vesal, 2005. Effect of tail fat on halothane biotransformation in fat-tailed sheep. *Clin. Exp. Pharmacol. Physiol.*, 32: 531-535.
- Spracklin, D.K., K.E. Thummel and E.D. Kharasch, 1996. Human reductive halothane metabolism *in vitro* is catalyzed by cytochrome P450 2A6 and 3A4. *Drug Metab. Dispos.*, 24: 976-983.
- Stone, E.A., J.L. Robertson and M.R. Metcalf, 2002. The effect of nephrotomy on renal function and morphology in dogs. *Vet. Surg.*, 31: 391-397.
- Thurmon, J.C., J. William, W.J. Tranquilli and G.J. Benson, 1996. Lumb and Jones Veterinary Anesthesia. 3rd Edn. Lippincott Williams and Wilkins, Philadelphia, ISBN: 0683082388 pp: 35- 36, 312-322.
- Topal, A., N. Gul, Y. Ilcol and O.S. Gorgul, 2003. Hepatic effects of halothane, isoflurane or sevoflurane anaesthesia in dogs. *J. Vet. Med. A Physiol. Pathol. Clin. Med.*, 50: 530-533.
- Uetrecht, J.P., 1997. Current trends in drug-induced autoimmunity. *Toxicology*, 119: 37-43.
- Wickstroem, I. and T. Stefansson, 1981. Effects of prolonged anesthesia with enflurane or halothane on renal function in dogs. *Acta Anaesthesiol. Scand.*, 25: 228-234.