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 nephrectomy on 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, Creatinine and Serum Fluoride Concentration (SFC) during the study. Following the first halothane anesthesia, BUN and Creatinine were significantly increased from 24 to 48 h compared to prenephrectomy, postnephrectomy and pranaesthesia in the second stage of the experiment, the significantly increases in BUN and Creatinine in comparison to prenephrectomy, postnephrectomy and pranaesthesia from 24 to 72 h and significantly increase in SFC concentration observed 3 to 72 h after anesethia in comparison to prenephrectomy, postnephrectomy and pranaesthesia.

Key words: Unilateral nephrectomy, halothane, dog

INTRODUCTION

Inhalant anesthetics are used widely for producing general anesthesia 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 anesthesia 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 Vesali, 2005); trifluoroethylene, 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 (Khanisch et al., 1995) halothane enflurane isofluorane (Klarasch 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 (Elcott and Strunin, 2003), the blood concentration of anesthetic (Sharifi and Vesali, 2005), genetic factors and sex (Sharifi and Vesali, 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.

Mozze et al. (2000) have been shown 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±SD) were used in this study. The dogs were confined to indoor camel 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 tranquillizers 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 mEq/L kg⁻¹) 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 prenephrectomy 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 weeks later post-nephrectomized dogs. Following the first halothane anesthesia, SFCs were not significantly increased from 3 to 27 h post-anesthesia compared to pre-anesthesia, prenephrectomy 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, prenephrectomy 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 (μmol/L) during in the first stage of experiment

<table>
<thead>
<tr>
<th>Blood biochemistry</th>
<th>Time of blood sampling</th>
<th>First part of anaesthesia</th>
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<td></td>
<td>Before nephrectomy</td>
<td>After nephrectomy</td>
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<td>Before nephrectomy</td>
<td>3h After nephrectomy</td>
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<td>6h After nephrectomy</td>
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<td>48h After nephrectomy</td>
<td>72h After nephrectomy</td>
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<tr>
<td>BUN</td>
<td>7.36±4.167</td>
<td>5.59±4.373</td>
</tr>
<tr>
<td>Creatinin</td>
<td>0.12±0.067</td>
<td>0.11±0.030</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.09±0.017</td>
<td>0.07±0.011</td>
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Table 2. Serum Fluoride concentration (ppm), BUN and Creatinin (μmol/L) during in the second stage of experiment

<table>
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<th>Blood biochemistry</th>
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Data are given as Mean±SD. Significant difference of BUN prenephrectomy, postnephrectomy and preanesthesia from baseline (p<0.05).

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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 pикnosis

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 premedications 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 ketamine. These effects following increase of fluoride ion to 50-80 μmol L⁻¹ started and when it reaches to 80-175 μmol L⁻¹ renal toxicity is completely obvious (Cousins et al., 1976).

Clinical studies with methoxyflurane showed that concentrations more than 50 μmol 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 μmol 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 Vesa, 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 (Purnford et al., 1993; Moul 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 (Uetrecht, 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 (Belnia 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 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


