RESEARCH ARTICLES

EFFECTS OF REPEATED SEVOFLURANE ANESTHESIA ON RENAL FUNCTION: AN ANIMAL STUDY
TEKRARLI SEVOFLURAN ANESTEZİSİNİN RENAL FONKSİYONLAR ÜZERİNE ETKİLERİ: DENEYSEL ÇALIŞMA

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ABSTRACT

Purpose: The effect of repeated administrations of sevoflurane in rats was evaluated with free inorganic plasma fluoride concentrations and the effect of sevoflurane on the kidneys. **Methods:** Thirty-five rats were divided into two groups. The control group consisted of 7 rats, and the sevoflurane group consisted of 28 rats. The sevoflurane group was divided equally into 4 subgroups according to the time of sacrifice (S1, S3, S5, S10). All rats in the sevoflurane group were administered 3% sevoflurane for 30 minutes for 5 days. After anesthesia, the rats were sacrificed at the end of the 1st, 3rd and 5th days. The other 7 were kept without anesthesia for 5 days and they were sacrificed at the end of the 10th day. Plasma inorganic fluoride concentrations were measured by ion-selective electrode methods in aspirated heart blood samples. Renal biopsies were taken just after sacrifice and the renal histopathologic effects of sevoflurane were investigated under light and transmission electron microscopy (TEM). **Results:** Plasma fluoride concentrations were significantly different between the sevoflurane and control groups. However, there was no statistical difference between the sevoflurane subgroups. Plasma BUN levels did not differ between the control and sevoflurane groups, except on the 10th day. Ultrastructurally, the sevoflurane subgroups (S-3, S-5, S-10) showed significant tubular histopathologic changes compared to the control group. However, these changes showed a significant regression on the 10th day. **Conclusion:** Repeated sevoflurane administration is a safe procedure since histopathological changes showed significant regression.

Key Words: Inhalation Anesthesia, Sevoflurane; Fluoride, Renal Toxicity.

ÖZET

Amaç: Bu çalışmada, ratlarda sevofluranın tekrarlı uygulamaları sonucunda, sevofluranın plazma inorganik flor düzeyleri ve renal sistem üzerine olan etkileri incelendi. **Metod:** Çalışmada 35 rat iki gruba ayrıldı (kontrol-sevofluran). Kontrol grubu 7 rattan ve sevofluran grubu 28 rattan oluşuyordu. Sevofluran grubu, ratların sakrifiye edilme zamanına gore herbiri 7 rat içeren 4 subgruba ayrıldı (S1, S3, S5, S10). Sevofluran grubuna ait tüm ratlara, 5 gün 30 dakika süreyle % 3 konsantrasyonda sevofluran anestezisi uygulandı. Ratlar 1., 3. ve 5. gün anestezi aldıktan sonra sakrifiye edildi. Kalan 7 rat, 5 gün anestezi almadan bekletildi ve 10. gün sakrifiye edildi. Plazma inorganik florid konsantrasyonu aspire edilen kalp kanından, iyon selektif elektrot metodu ile ölçüldü. Ratlardan böbrek biyopsileri sakrifiye edildikten hemen sonra alındı. Sevofluranın renal histopatolojik etkileri ışık ve transmisyon elektron mikroskopisi ile değerlendirildi (TEM). **Bulgular:** Plazma inorganik flor konsantrasyonları her iki grup arasında anlamlı farklıydı. Buna karşın sevofluran subgrupları arasında fark yoktu. Her iki grup arasında plazma BUN ve kreatinin düzeyleri arasında fark yok iken, yalnızca 10. gündeki plazma BUN düzeyleri farklı bulundu. Elektromikroskobik görünüm olarak sevofluran subgrupları ile kontrol grubu arasında anlamlı tübüler değişiklikler izlendi. Ancak bu değişiklikler 10. günde belirgin regresyon gösterdi. **Tartışma:** Sonuç olarak, histopatolojik değişiklikler reversibl olduğu için, bizim metodumuzla tekrarlı sevofluran uygulamasının güvenilir olduğu sonucuna varıldı.

Anahtar Kelimeler: İnhalasyon Anestezisi, Sevoflurane; Flor, Renal Toksisite.

INTRODUCTION

Biotransformation of fluorinated ether volatile anesthetics results in the production of fluoride metabolites capable of producing hepatic or renal toxicity. Inorganic fluoride ions, liberated during the metabolism of certain agents, may

cause subclinical nephrotoxicity or overt renal insufficiency at excessive concentrations (1). Fluoride ion is a potent inhibitor of metabolic processes. Sevoflurane is a rapid-acting potent inhaled anaesthetic and it is biotransformed to organic and inorganic fluoride metabolites. Sevoflurane undergoes oxidative defluorinization with liberation of free fluoride ion. The additional major metabolites of sevoflurane have been well characterized (2). Biotransformation of sevoflurane to inorganic fluoride is mainly performed by cytochrome 450 2E1 (P 450 2E1) in the liver (3). It was indicated that inorganic fluoride and hexafluoroisopropanol (HFIP) were major products of the human sevoflurane metabolism. HFIP circulates in the blood primarily as the glucuronide conjugate and is excreted in urine. Clinical evaluations of sevoflurane have shown wide variability in metabolism, as monitored by plasma fluoride concentrations. Average peak plasma fluoride concentrations ranged from 15 to 30 μM after 1-2 MAC sevoflurane (4). Higher plasma fluoride concentrations have been associated with longer sevoflurane exposures. A positive correlation between the amount of renal exposure to inorganic fluoride and variables showing renal damage has been reported (5-7). Studies performed with sevoflurane usually involve long exposure times (> 4 h). After the application, the serum inorganic F-level and effects on renal function are examined (8, 9).

In the present study, to investigate the renal toxicity of sevoflurane the question Is sevoflurane toxic when administered in the same concentration for short applications (30 min) repeatedly? is asked. We evaluated the effects of repeated sevoflurane anesthesia on plasma inorganic fluoride concentrations, BUN and creatinine levels in animal models. The renal histopathologic effects of sevoflurane were also investigated under light and transmission electron microscopy.

MATERIALS AND METHODS

This study was performed on 35 male Sprague-Dawley rats weighing 250-300 g. All animals were kept at room temperature and fed ad libitum. The rats were divided into two groups (control and sevoflurane). The control group contained 7 rats and the sevoflurane group 28 rats. The sevoflurane group was divided into 4 equal subgroups according to the times of sacrifice (S1, S3, S5, S10). We sacrificed the rats in the subgroups on the 1st day (S1), 3rd day (S3), 5th day (S5) and 10th day (S10). Before the study, anesthesia calibration was performed with gas chromatographic control and 1.3 MAC/h sevoflurane was found to be equal to a 3% concentration of sevoflurane.

The rats were placed in desiccators and anesthetized with a 3% concentration of sevoflurane in 6 L/min O_2 for 30 minutes using a Pediatric Circuit System, Chirana Anesthesia Machine and Sevotec temperature-compensated vaporizer. In this model soda lime was not used. All rats in the control group (n=7) were anesthetized with ketamine IM and were sacrificed immediately after anesthesia. The rats in the sevoflurane group (n=28) were given sevoflurane for 30 minutes at a concentration of 3%; just after this anesthesia 7 of them were sacrificed and they formed subgroup S1. On the second day, the remaining 21 rats were again given 30 minutes of anesthesia with a 3% concentration of sevoflurane. On the third day, 21 rats were given anesthesia in exactly the same way and 7 of them were sacrificed after this anesthesia and they formed subgroup S3. On the fourth day, the remaining 14 rats were again given anesthesia at the same dose and in the same way. The same procedure was repeated on the fifth day, but was followed by the sacrifice of 7 rats to form subgroup S5. Finally, the remaining 7 rats were not exposed to anesthesia for 5 days and were sacrificed on the 10th day. This group is named subgroup S10.

All rats were sacrificed by total aspiration of blood from the heart. Five milliliters of blood was preserved to measure the plasma fluoride concentration and for renal function tests. Plasma fluoride concentrations were determined using an ion-selective electrode. One milliliter of standard fluoride solution or serum was pipetted into a polyethylene cell equipped with a stirring bar. Then 0.01 ml of acetate buffer prepared by mixing equal volumes of 10 M acetic and 5 M sodium hydroxide was added. Fluoride concentrations were measured using an Orion 720A pH meter in combination with a fluoride electrode model Orion 96-09 BN. Potential measurements were taken every minute until a constant reading was attained, usually within 2 to
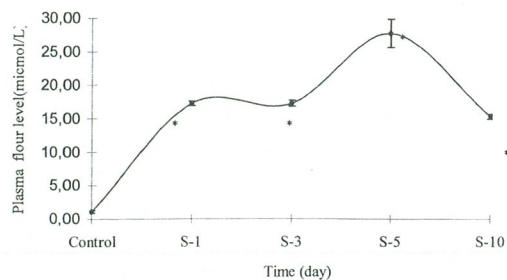
5 minutes. Calibration curves of fluoride were prepared by diluting a standard 1.0 M sodium fluoride solution with isotonic saline.

Kidney biopsies were taken immediately after the aspiration of blood. The tissue samples were fixed with 2.5% phosphate buffer in glutaraldehyde and 2% paraformaldehyde solution for 1 day, then washed in Seronson buffer solution at +4 °C and buried in Araldite material. Sections from these materials were dyed with toluidine blue and examined by light microscope. Tissue samples were also thinned to 1-2 using an LKB Nova Ultratom and examined by JEOL JEM 1200 Electron Microscope.

The Kruskal-Wallis one-way ANOVA was used to compare the plasma fluoride, creatinine and BUN levels of the groups, and then the Mann-Whitney U test was used to determine the differences between the groups. All results are expressed as mean – SD. Statistical analysis was performed using SPSS 9.0 software. $p < 0.05$ was considered statistically significant.

RESULTS

Plasma inorganic fluoride concentrations were 17.2 – 0.3 µM/L on the 1st day, 17.2 – 0.4 on the 3rd day, 25.7 – 2.1 µM/L on the 5th day, and 15.3 – 0.3 µM/L on the 10th day. It was 1.1 – 0.2 µM/L in the control group. There were statistically significant differences between all sevoflurane and control groups (p=0.0028, Fig. 1). However, there were no statistically significant differences between the sevoflurane groups (p=0.675). Plasma BUN levels did not differ between the control and sevoflurane groups, except on the 10th day. The value of BUN was found to be increased on the 10th day (p<0.05, Fig. 2). Plasma creatinine levels did not differ between the sevoflurane and control groups (p>0.05, Fig. 3).

Histopathologic examination:

The histopathologic examinations of renal biopsies of the control group were normal (Fig. 4a). The histopathologic examinations of specimens on the 1st day revealed seldom local edema at the proximal tubules, both under light microscopy and EM (Fig. 4b). However, glomerular structures were normal (Fig. 4c). In the 3rd and 5th days' specimens, there was a decrease in the number of mitochondria in tubular cells and lysosomal vacuolization in the
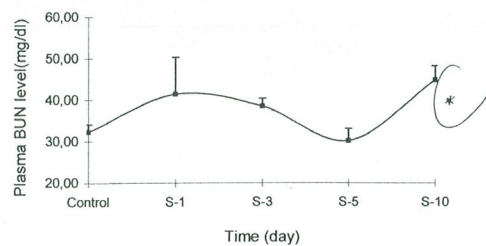
* p<0.05, compared to control.
Fig. 2: Plasma BUN levels of the groups.

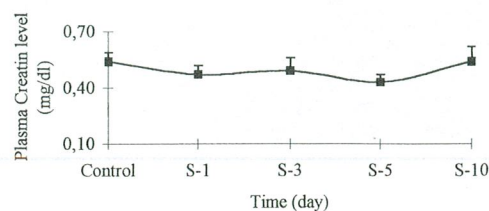* p<0.05, compared to control.
Fig. 1: Plasma inorganic fluoride levels of the groups.
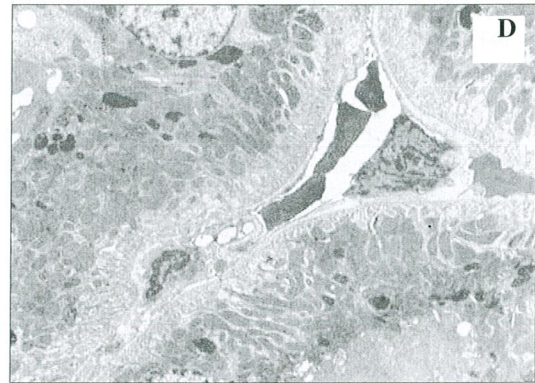
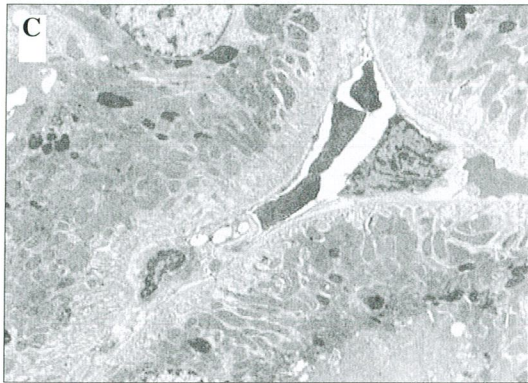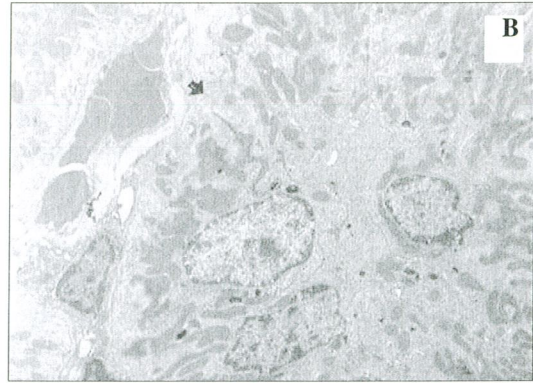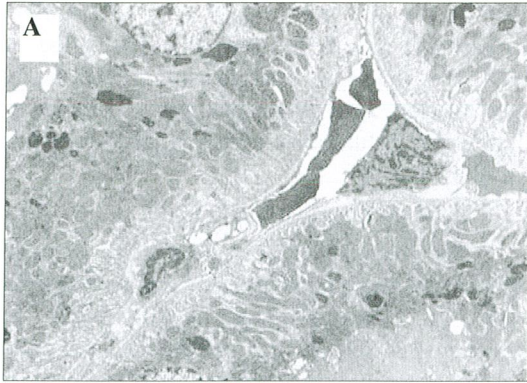Fig. 3: Plasma creatinine levels of the groups.

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Fig. 4: The normal histopathologic appearance with TEM, in the control group (A). The histopathologic appearance with TEM on the 1st day (B). The normal glomerular histopathologic structure on the 1st day (C). The histopathologic appearance with TEM on the 5th day (D).
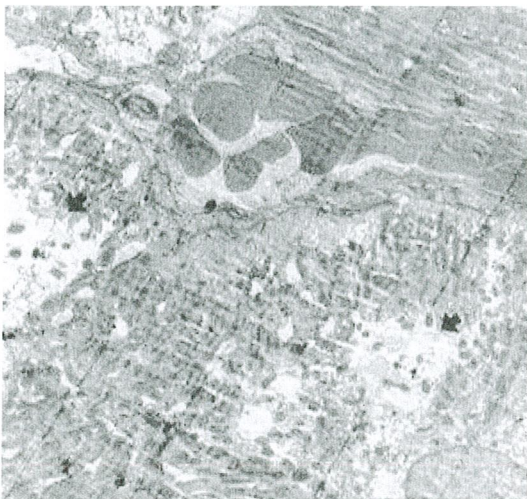

Fig. 4E: The histopathologic appearance with TEM on the 10th day.

cytoplasm (Fig. 4d). On the 10th day, a significant regression was observed in the histopathologic tubular changes (Fig. 4e).

DISCUSSION

In the present study we investigated the inorganic fluoride kinetics and renal function with renal histopathologic changes after "repeated short duration exposure" to sevoflurane in rats.

Currently used fluorinated anesthetics are chemically related to methoxyflurane, a drug that caused many cases of clinical acute renal failure during previous widespread use. In a study using metoxyflurane, isoflurane and sevoflurane in rats, it was observed that intracellular ATP consumption and inhibition of Na-K-ATP'ase could be the mechanism of fluoride toxicity (10). Fluorinated anesthetic-mediated proximal tubular

injuries are likely to be a mechanism contributing to ATP depletion and Na-K-ATP'ase inhibition. Fluoride ion is the major determinant of this toxicity, and tubular injury can be expressed at or near clinically relevant anesthetic/inorganic fluoride levels (10). Nuscher et al. noted that the concentration of inorganic fluoride from metabolized sevoflurane was affected by various factors such as total amount of anesthetic agent, its solubility and blood/gas partition ratio (11).

Although in many studies sevoflurane has been given for long durations (4, 9, 12, 13), studies of the same anesthetic given repeatedly are few (14-16). Two of these are human studies in which the patients received sevoflurane with an interval of 30-90/30-180 days (14, 15). In our study we investigated the effects of repeated exposure to sevoflurane in rats, which is not very suitable for a volunteer human model.

After prolonged sevoflurane exposure (average 4.7 MAC.h), the serum peak inorganic fluoride value is known to be 50 μMol/L, and it is reported that there is a correlation between the increase in serum inorganic fluoride levels and the duration of sevoflurane anesthesia (3-5, 9, 17). The degree of renal tubular damage correlated well with the inorganic fluoride levels (5). Some studies showed that serum fluoride concentrations decrease to about 50% of the peak within 8-12 h of anesthetic discontinuation (4, 5, 12). This rapid decrease in plasma fluoride concentration appears to be due to the rapid elimination of sevoflurane.

Renal dysfunction in humans has not been reported to occur in prolonged sevoflurane anesthesia (15 MAC/h) (12). Kobayashi et al. showed that after 13.5 MAC/h of sevoflurane anesthesia the levels of BUN and creatinine did not change, indicating no neprotoxicity (13). Another study, by Frink et al., also confirmed the previous study; they found the levels of inorganic fluoride to be high 6 hours postoperatively. However, this tended to decrease after 12 hours. In addition, BUN and creatinine levels were reported to be normal on the 1st, 4th and 5th days of application (9). Furthermore, in some studies, it was reported that the duration of sevoflurane administration and the area under the curve for serum fluoride did not affect renal function (8, 18).

Recent studies showed that there were no significant changes in the renal and hepatic functions of patients who had received a second administration of sevoflurane anesthesia (14, 15). It is reported that there were no significant differences in BUN, creatinine, serum fluoride levels, total bilirubin, serum concentrations of liver enzymes or urinary excretion of protein between the first and second anesthesia. These two studies about repeated exposure to sevoflurane showed no additional risk of increasing renal or hepatic injury. However, in these studies, the period between the first and second anesthesia was at least 30 days. Therefore, the long period may have caused these results.

In our study, 3% sevoflurane was applied for 1, 3 and 5 days, each day for 30 minutes. By doing so, we aimed to keep the serum fluoride concentrations elevated. Administration of sevoflurane in a circle absorption system has been shown to generate Compound A, a nephrotoxin in rats (18, 19). As soda lime (CO_2 absorption) was not used in our study, the formation and toxic effects of Compound A were ignored. The peak plasma fluoride level was significantly higher in the sevoflurane groups than in the control group. There was a 53.12% decrease between the serum fluoride level in subgroup S5 on day 5 and the serum fluoride level in subgroup S10 on day 10. The serum fluoride concentration was expected to return to the basal level due to the rapid elimination of sevoflurane. The decrease in the serum fluoride concentration in the present study cannot be explained by the metabolism of sevoflurane.

In our study, BUN levels increased on the 10th day only. This may be related to the renal function of rats, which was influenced by the feeding and shelter conditions, and the rats may also have been hypotensive during the postanesthesia period, affecting the results. The classical histopatologic changes of prerenal cause are tubular intertisial edema, and tubular and papillary necrosis. We think that these histopatologic changes were not related to increased BUN levels because of the significant regression in the histopathologic changes observed in the rats on the 10th day.

In conclusion, a declining inorganic fluoride ion tendency was observed when the rats were exposed to sevoflurane repeatedly, but the level of inorganic fluoride ion did not return to the

basal level. In addition, on the 10th day, a significant regression was observed in the histopathologic tubular changes. Based on these results, we think that the 50% decrease in the plasma fluoride level was significant and both the regression in the histopathologic changes and the 50% decrease in the plasma fluoride level were contributing factors.

We conclude that repeated sevoflurane administration is a safe procedure when nephrotoxicity is concerned because histopathologic changes are reversible.

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REFERENCES

1. Cousins MJ, Mazze RI. Metoxyflurane neprotoxicity in man. JAMA 1973; 225: 1611-1616.
2. Kharash ED, Karol MD, Lanni C. Clinical sevoflurane metabolism and disposition. Sevoflurane and metabolite pharmacokinetics. Anesthesiology 1995; 82: 1369-1378.
3. Gentz BA, Malan TP. Renal toxicity with sevoflurane: a strom in a teacup? Drugs 2001; 61: 2155-62.
4. Frink EJ, Ghantous H, Malan TP, Morgan S, Fernando J, Gandolfi AJ, Brown BR . Plasma inorganic fluoride with sevoflurane anesthesia: Correlation with indices of hepatic and renal function. Anesth Analg 1992; 74: 231-235.
5. Nishiyama T, Toda N. Correlation between renal function and pharmacokinetic paramethers of inorganic fluoride following sevoflurane anaesthesia. J Anaesth 1995; 77: 125-128.
6. Mazze RI. Fluorinated anesthetic neprotoxicity: an update. Can J Anaest Soc J 1984; 31(Supp): 16-22.
7. Norgate CE, Sharp JH, Cousins MJ. Metabolism of enflurane in man following a second exposure. Anaesht Intensive Care 1976; 4: 186-191.
8. Higuchi H, Arimura S, Sumikura H, Satch T, Kanno M. Urine concentrating ability after prolonged sevoflurane anaesthesia. Br J Anaesth 1994; 73: 239-240.
9. Frink EJ, Malan P, Isner J, Brown E, Morgan SE, Brown BR. Renal concentrating function with prolonged sevoflurane or enflurane anesthesia in volunteers. Anesthesiology 1994; 80: 1019-1025.
10. Lochhead KM, Kharash ED, Zager RA. Spectrum and subcellular determinants of fluorinated anesthetic-mediated proximal tubular injury. Am J Pathol 1997; 150: 2209-2221.
11. Nuscher M, Conzen P, Peter K. Sevoflurane metabolism and toxicity. Anaesthesist 1998; 47: 24-32.
12. Kazama T, Ikeda K. The effect of prolonged administration of sevoflurane on serum concentration of fluoride ion in patients. Anesthesiology 1991; 75: 53-56.
13. Kobayaschi Y, Ochiai R, Takeda J, Sekiguchi H, Fukushima K. Serum and urinary fluoride concentrations after prolonged inhalation of sevoflurane in humans. Anesth Analg 1992; 74: 753-757.
14. Nishiyama T, Hanaoka K. Inorganic fluoride kinetics and renal and hepatic function after repeated sevoflurane or isoflurane anesthesia. Anesth Analg 1998; 87: 468-473.
15. Nishiyama T, Yokoyama T, Hanaoka K. Liver and renal function after repeated sevoflurane or isoflurane anaesthesia. Can J Anaesth 1998; 45: 789-793.
16. Soma LR, Tierney WJ, Hogan GK, Satoh N. The effects of multiple administrations of sevoflurane to cynomolgus monkeys: clinical pathologic, hematologic and pathologic study. Anesth Analg 1995; 81: 347-352.
17. Malan TP, Kadota Y, Mata H. Renal function after sevoflurane or enflurane anesthesia in the Fisher 344 rat. Anesth Analg 1993; 77: 817-821.
18. Goldberg ME, Cantillo J, Gratz I, Đeal E, Vekeman D, McDougall R, Afshar M, Zaferidis A, Larijani G. Dose of compound A, not sevoflurane, determines changes in biochemical markers of renal injury in healthy volunteers. Anesth Analg 1999; 88: 437-445.
19. Catania JM, Parrish AR, Gandolfi AJ. Toxicity of sevoflurane degradation product incubated with rat liver and renal cortical slices. Drug and Chemical Toxicology 2001; 24: 347-357.