Fluoride metabolism in smokers and non-smokers following enflurane anaesthesia

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Background. Inorganic fluoride is released by the metabolism of enflurane and the increased serum fluoride concentrations may impair renal function. Tobacco smoke consists of numerous reactive compounds that can either induce or inhibit drug metabolism. Studies on the interaction of smoking with anaesthetic drug metabolism and possible toxicity are warranted.

Methods. Sixteen non-smoking and 17 smoking (>10 cigarettes day−1) generally healthy women undergoing elective gynaecological surgery were given 1 MAC (minimum alveolar concentration)-hour standardized anaesthesia with enflurane in oxygen–air mixture. The serum inorganic fluoride and renal function markers β2-microglobulin, tumour-associated trypsin inhibitor (TATI) and serum creatinine were measured for 48 h.

Results. The greatest inorganic fluoride concentration was between 8.4 and 21.0 (mean 13.8 (SD 3.4)) μmol litre−1 in the non-smokers and between 8.6 and 38.0 (18.7 (7.0)) μmol litre−1 in the smokers; the mean difference was 4.9 μmol litre−1 (95% confidence interval (CI) 1.0–8.8, P<0.05). Serum β2-microglobulin, TATI and creatinine were not increased. Serum inorganic fluoride concentrations were significantly greater in the smokers compared with the non-smokers 1, 2, 3 and 6 h after 1 MAC-hour inhalation with enflurane (P<0.05). Inorganic fluoride concentrations were still increased 24 h after anaesthesia in both groups. Urine β2-microglobulin and TATI creatinine ratio remained at low values during the whole 48-h period in both groups.

Conclusions. Regular smoking is associated with an increase in serum inorganic fluoride concentration after anaesthesia with enflurane, but there are no signs of renal damage.

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Metabolism of enflurane releases inorganic fluoride but does not appear to be harmful in patients with normal renal function. Rarely inorganic fluoride concentrations may reach the suggested renal toxic threshold (50 μmol litre−1),1 but impaired renal function has been reported after prolonged enflurane anaesthesia.2 Serum fluoride concentration during enflurane anaesthesia can be affected by other therapy. For example, concomitant treatment with isoniazid (inducer of hepatic drug metabolism) increases serum fluoride concentrations3 while disulfiram (inhibitor of drug metabolism) reduces fluoride concentrations.4 Enzyme induction by aminoglycosides can increase the nephrotoxicity of enflurane, as indicated by excretion of urinary alanine aminopeptidase as a marker of proximal tubular function.5 Smoking is increasing among Finnish women,6 but little is known about the interaction of smoking with anaesthetic drugs. Tobacco smoke contains numerous components, some of which have been investigated regarding their
pharmacological effects on the body. For example, nicotine and its main metabolite inhibit cytochrome P450 2E1 (CYP2E1), the isoenzyme responsible for defluorination of fluorinated ether anaesthetics. In addition, the polyaromatic hydrocarbons found in tobacco smoke are potent inducers of drug metabolism and induction of liver enzymes in smokers increases the metabolism of antipyrine, pentazocine and theophylline. We noted that plasma inorganic fluoride concentrations seemed to be greater in smokers than in non-smokers after enflurane anaesthesia. Because our knowledge of fluoride metabolism in smokers is sparse, we designed this prospective clinical trial to determine whether smoking tobacco influences serum concentrations of inorganic fluoride after enflurane anaesthesia. Serum creatinine, $\beta_2$-microglobulin and tumour-associated trypsin inhibitor (TATI) were used to evaluate possible renal damage.

Materials and methods

The study was approved by the ethics committee of the Women’s Hospital, University of Helsinki and was conducted in accordance with the Declaration of Helsinki. Thirty-three women (ASA I–II) without pre-existing renal disease were enrolled. None of the patients used any concomitant medication known to modify cytochrome P450 activity. Seventeen smokers (more than 10 cigarettes daily) and 16 non-smokers underwent elective laparoscopic or open abdominal gynaecological operations. All patients were informed and gave written consent.

Anaesthesia was standardized. Diazepam 5–10 mg orally was used for premedication. The patients were given glycopyrrolate 0.2 mg and fentanyl 0.1–0.15 mg i.v., anaesthesia was induced with propofol 2.5 mg kg$^{-1}$ i.v., and tracheal intubation was facilitated with rocuronium 0.5 mg kg$^{-1}$ i.v.

Anaesthesia was maintained with 1.3 minimum alveolar concentration (MAC) of enflurane (the end-tidal concentration of enflurane was set to 2.1%) in 33% oxygen–air mixture for 45 min, which corresponds to a 1 MAC-hour anaesthesia. A semiclosed breathing system with fresh gas flow of 3 litres min$^{-1}$ was used. The concentrations of inhalation gases and anaesthetics were monitored with a Datex AS/3 monitor (Datex-Ohmeda, Instrument Corporation, Helsinki, Finland). The inhalation anaesthesia was discontinued after 45 min. Thus, all patients in both study groups were given 1 MAC-hour inhalation anaesthesia with enflurane.

After cessation of enflurane administration, the anaesthesia was continued with propofol infusion and the lungs were ventilated with oxygen 33% in nitrous oxide. Additional doses of fentanyl and neuromuscular blocking drug were given when needed.

Fluid therapy was standardized for the first 24 h. Patients were given Ringer’s solution 10 ml kg$^{-1}$ at induction of anaesthesia followed by an infusion of 5 ml kg$^{-1}$ h$^{-1}$ during surgery and in the recovery room. From the first post-operative day patients were allowed to drink *ad libitum*.

Venous blood samples for serum inorganic fluoride were collected before anaesthesia and 1, 2, 3, 6 and 24 h after the end of enflurane inhalation. Serum and urine samples for creatinine, $\beta_2$-microglobulin and TATI were collected at induction and on the first and second postoperative mornings. $\beta_2$-Microglobulin is a marker of renal tubular damage and TATI of glomerular filtration rate. The urinary catheter was inserted after the induction of anaesthesia for urine sampling. The urine markers were measured from spot samples to prevent the confounding effect of different urine volumes. The urinary catheter was removed when it was no longer clinically necessary.

The concentrations of inorganic fluoride were determined by a method modified from that of Fry and Taves. In brief, a fluoride-selective combination electrode (Orion model 96-09; Orion Research, Boston, MA, USA) was used for the measurement on Parafilm M (American National Can, Greenwich, CT, USA) placed on 16-mm cell culture wells. Before measurement, 200 $\mu$L of acetate buffer (acetate–NaOH 1 mol litre$^{-1}$, pH 5.2, NaCl 1 mol litre$^{-1}$) and 10 $\mu$L of sodium fluoride 20 $\mu$mol litre$^{-1}$ were added to 190 $\mu$L of serum. The sensitivity of this assay was approximately 0.5 $\mu$mol litre$^{-1}$ and the interassay coefficient of variation was less than 8%.

The $\beta_2$-microglobulin concentration was determined using time-resolved fluoroimmunoassay (Delfia; Wallac, Turku, Finland). Urine TATI was analysed by radioimmunoassay (Orion Diagnostica, Espoo, Finland). Serum and urine creatinine were analysed using a kinetic Jaffe method. The reference limits of the hospital laboratory are for serum TATI 0–2 nmol litre$^{-1}$, for urine TATI indexed to sodium fluoride 20 $\mu$mol litre$^{-1}$ and less than 0.25 mg litre$^{-1}$.

Statistics

We estimated the required sample size from our pilot observations, which found that the mean serum inorganic fluoride concentration was 12 (SD 4) $\mu$mol litre$^{-1}$ after enflurane anaesthesia in non-smoking women. From these data we estimated that 15 patients in each group would provide 80% power to detect a 35% difference in inorganic fluoride concentrations between the groups with a 0.05 level of significance.

The area under the serum fluoride concentration time curve from zero time to the 24 h blood sampling (AUC$_{0\rightarrow t}$ $\mu$mol litre$^{-1}$) was calculated according to the linear trapezoidal rule.

Statistical analyses were with SPSS 9.0 (SPSS, Chicago, IL, USA). The $\chi^2$-test was used for categorical variables. The independent samples two-tailed $t$-test was used to analyse values with a normal distribution (tested with Leaven’s test), and for skewed data we used the Mann–
The greatest inorganic fluoride concentration ranged between 8.4 and 21.0 (mean 13.8 (SD 3.4)) µmol litre⁻¹ in the non-smokers and between 8.6 and 38.0 (18.7 (7.0)) µmol litre⁻¹ in the smokers (mean difference 4.9 µmol litre⁻¹; 95% confidence interval (CI) 1.0–8.8 µmol litre⁻¹; *P*<0.01, Mann–Whitney *U*-test). The AUC of serum fluoride concentration ranged between 119 and 347 (217 (65) µmol h litre⁻¹) in the non-smokers and between 119 and 616 µmol h litre⁻¹ (318 (130) µmol h litre⁻¹) in the smokers (mean difference 33.6 µmol h litre⁻¹; 95% CI 12–165 µmol h litre⁻¹; **P**<0.001, Mann–Whitney *U*-test). The interaction between the components of tobacco smoke and anaesthetic inhalation agents is poorly investigated. Enflurane is predominantly metabolized in the liver by cytochrome P450 2E1 isoform to inorganic fluoride.\(^4\) Tobacco smoke contains powerful inducers of cytochrome P450 enzymes and the induction of cytochrome P450 2E1 is the most likely reason for increased concentrations of inorganic fluoride after enflurane anaesthesia. However, interactions between the components of tobacco smoke and anaesthetic inhalation agents is poorly investigated.

Inorganic fluoride concentrations seldom reach the renal toxic concentration of 50 µmol litre⁻¹ during short inhalation anaesthesia.\(^1\) In volunteers renal concentrating ability decreased when serum inorganic fluoride was maximal at only 33.6 µmol litre⁻¹ after enflurane anaesthesia.\(^18\) In the present study, one woman in the smokers group had a serum fluoride concentration of 38 µmol litre⁻¹ while the greatest concentration in the non-smokers group was 21 µmol litre⁻¹.

It is probable that it is not the maximum concentration but rather the duration of raised inorganic concentrations (i.e. the area under the curve) which may affect the development of nephrotoxic effects after enflurane anaesthesia.\(^19\) Also, other reactive intermediate metabolites may be potential nephrotoxins. For example, alkaline degradation of enflurane produces halogenated alkenes that are conjugated further to possibly nephrotoxic thiol compounds.\(^20\)

Renal tubular function remained stable in this study, as indicated by serum and urine β₂-microglobulin concentra-
Table 2 Renal markers. Data are mean (SD)

<table>
<thead>
<tr>
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<th>Serum creatinine (μmol litre⁻¹)</th>
<th>Serum TATI (nmol litre⁻¹)</th>
<th>Urinary TATI/creatinine (nmol mmol⁻¹)</th>
<th>Serum μ₂-microglobulin (mg litre⁻¹)</th>
<th>Urinary μ₂-microglobulin/creatinine (mg mmol⁻¹)</th>
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<tbody>
<tr>
<td><strong>Non-smokers</strong></td>
<td></td>
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<tr>
<td>Baseline (n=6)</td>
<td>78 (6)</td>
<td>1.0 (0.3)</td>
<td>0.5 (0.4)</td>
<td>1.6 (0.3)</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td>1st postoperative day (n=16)</td>
<td>71 (8)</td>
<td>1.2 (0.6)</td>
<td>0.8 (0.7)</td>
<td>1.3 (0.3)</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>2nd postoperative day (n=16)</td>
<td>75 (7)</td>
<td>1.9 (1.0)</td>
<td>1.1 (1.0)</td>
<td>1.5 (0.3)</td>
<td>0.03 (0.05)</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (n=16)</td>
<td>74 (9)</td>
<td>1.6 (1.1)</td>
<td>0.8 (1.2)</td>
<td>1.5 (0.3)</td>
<td>0.02 (0.03)</td>
</tr>
<tr>
<td>1st postoperative day (n=16)</td>
<td>73 (8)</td>
<td>2.0 (1.8)</td>
<td>1.2 (1.7)</td>
<td>1.2 (0.4)</td>
<td>0.03 (0.03)</td>
</tr>
<tr>
<td>2nd postoperative day (n=11)</td>
<td>73 (6)</td>
<td>1.6 (1.1)</td>
<td>0.7 (0.5)</td>
<td>1.3 (0.3)</td>
<td>0.02 (0.02)</td>
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tions. The β₂-microglobulin is a low molecular weight protein produced predominantly by lymphocytes.¹⁴ It is filtered through the glomerulus and reabsorbed in the proximal tubules. Serum and urine β₂-microglobulin concentrations increase in patients with tubular damage. Increased serum concentrations of β₂-microglobulin have been measured in connection with high inorganic fluoride concentrations after sevoflurane anaesthesia.²¹ Serum concentrations of β₂-microglobulin are also influenced by immunological and stress reactions, and infections, because of the predominant production in lymphocytes. In our study, a possible influence of diurnal variation in serum β₂-microglobulin concentrations, with peak values in the morning, was minimized by sampling at the same time each morning. The urine markers were determined from spot samples and normalized to urinary creatinine. The use of spot samples may have affected the results, although this normalization to urine creatinine is commonly used to avoid the confusing effect of different urine volumes.¹⁶

A small amount of TATI is found in the serum and urine of healthy subjects, and serum TATI concentration has a negative correlation with glomerular filtration rate. If glomerular filtration rate is reduced, serum TATI increases with increased urinary excretion.¹⁵ In the present study the fluid therapy was standardized for the first 24 h after surgery and thereafter the patients were allowed to drink ad libitum. This may have affected the urinary marker results. TATI is used as a tumour marker for conditions such as ovarian cancer.²³ This could have been a confounding factor in the present study.

We conclude that inorganic fluoride concentrations are increased markedly in smokers compared with non-smokers after 1 MAC-hour enflurane anaesthesia. Induction of cytochrome P₄₅₀ enzymes by cigarette smoke is likely to be the cause. However, we did not find fluoride-induced nephrotoxicity.

References


3 Mazze RI, Woodruff RE, Heerdt ME. Isoniazid-induced enflurane defluorination in humans. Anesthesiology 1982; 57: 5–8


5 Motuz DJ, Watson WA, Barlow JC, Velasquez NV, Schentag JJ. The increase in urinary alanine aminopeptidase excretion associated with enflurane anesthesia is increased further by aminoglycosides. Anesth Analg 1988; 67: 770–4


8 Kharasch ED, Thummel KE. Identification of cytochrome P₄₅₀ 2E1 as the predominant enzyme catalysing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. Anesthesiology 1993; 79: 795–807


16 Wellwood JM, Ellis BG, Price RG, et al. Urinary N-acetyl-β-D-
17 Fry BW, Taves DR. Serum fluoride analysis with the fluoride electrode. J Lab Clin Med 1970; 75: 1020–5