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, β2-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 at 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 protopof 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, β2-microglobulin and TATI were collected at induction and on the first and second postoperative mornings. β2-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 µl of acetate buffer (acetate–NaOH 1 mol litre⁻¹, pH 5.2, NaCl 1 mol litre⁻¹) and 10 µl of sodium fluoride 20 µmol litre⁻¹ were added to 190 µl of serum. The sensitivity of this assay was approximately 0.5 µmol litre⁻¹ and the interassay coefficient of variation was less than 8%.

The β2-microglobulin concentration was determined using time-resolved fluoroimmunoassay (Delta®; 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 urinary creatinine 0–1.3 nmol mmol⁻¹ creatinine, and for serum and urine β2-microglobulin respectively 0.6–3.0 mg 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) µmol 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 (AUCₚᵣ) was calculated according to the linear trapezoidal rule.

Statistical analyses were with SPSS 9.0 (SPSS, Chicago, IL, USA). The χ²-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–
Table 1 Patient characteristics. Data are mean (range or sd) or number of cases

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers (n=16)</th>
<th>Smokers (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48 (27–58)</td>
<td>44 (35–39)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 (7)</td>
<td>166 (5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65 (6)</td>
<td>67 (10)</td>
</tr>
<tr>
<td>Duration of anaesthesia (min)</td>
<td>95 (27) (60–160)</td>
<td>110 (33) (50–200)</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparotomy</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Vaginal</td>
<td>2</td>
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Whitney U-test. The Friedman test was used to compare inorganic fluoride concentrations observed over the different sample times. A $P$-value of $\leq 0.05$ was considered statistically significant. The data are expressed as mean (SD) and minimum–maximum unless otherwise stated.

Results

The characteristics of the two groups are shown in Table 1. In the smokers group there were 13 moderate smokers (10–20 cigarettes per day) and four heavy smokers (more than 20 cigarettes per day). No patients were withdrawn from the study. There were some minor protocol deviations, which were unlikely to have interfered with the results; blood samples for the determination of inorganic fluoride were not obtained for one patient at baseline, for seven patients at 6 h and for seven patients at 24 h. All the other 183 samples were drawn according to the trial protocol.

Inorganic fluoride concentration increased significantly in both groups after 1 MAC-hour enflurane anaesthesia ($P<0.001$, Friedman test) and was still increased at 24 h in both study groups ($P<0.001$, paired sample $t$-test). However, in the smokers the fluoride concentration was significantly greater than in the non-smokers ($P<0.05$) 1, 2, 3 and 6 h after enflurane inhalation (Fig. 1).

The greatest inorganic fluoride concentration ranged between 8.4 and 21.0 (mean 13.8 (SD 3.4)) $\mu$mol litre$^{-1}$ in the non-smokers and between 8.6 and 38.0 (18.7 (7.0)) $\mu$mol litre$^{-1}$ in the smokers (mean difference 4.9 $\mu$mol litre$^{-1}$, 95% confidence interval (CI) 1.0–8.8 $\mu$mol litre$^{-1}$; $P=0.018$, Mann–Whitney $U$-test). The AUC of serum fluoride ranged between 131 and 347 $\mu$mol litre$^{-1}$ (217 (65) $\mu$mol litre$^{-1}$) in the non-smokers and between 119 and 616 $\mu$mol litre$^{-1}$ (318 (130) $\mu$mol litre$^{-1}$) in the smokers (mean difference 101 $\mu$mol litre$^{-1}$, 95% CI 12–190 $\mu$mol litre$^{-1}$; $P=0.028$, Mann–Whitney $U$-test).

Serum TATI and $\beta_2$-microglobulin values did not differ between the two groups. Urine TATI and the $\beta_2$-microglobulin–creatinine ratio remained at small values during the study period and serum creatinine remained at the baseline in both study groups during the first 2 postoperative days (Table 2).

Discussion

We found that the serum concentrations of inorganic fluoride were significantly greater in women who smoked compared with the non-smokers after 1 MAC-hour enflurane anaesthesia. The concentration of fluoride was greatest 2–3 h after anaesthesia and was still increased at 24 h in both study groups, although no significant difference between the two groups was present after 24 h. No nephrotoxic effects were found: there were no changes in renal tubular reabsorption capacity and glomerular filtration assessed by serum and urine $\beta_2$-microglobulin, TATI and serum creatinine measurements.

Enflurane is predominantly metabolized in the liver by cytochrome P450 2E1 isoenzyme to inorganic fluoride.\textsuperscript{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, the interaction between the components of tobacco smoke and anaesthetic inhalation agents is poorly investigated.

Inorganic fluoride concentrations seldom reach the renal toxic concentration of 50 $\mu$mol litre$^{-1}$ during short inhalation anaesthesia.\textsuperscript{1} In volunteers renal concentrating ability decreased when serum inorganic fluoride was maximal at only 33.6 $\mu$mol litre$^{-1}$ after enflurane anaesthesia.\textsuperscript{18} In the present study, one woman in the smokers group had a serum fluoride concentration of 38 $\mu$mol litre$^{-1}$ while the greatest concentration in the non-smokers group was 21 $\mu$mol 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.\textsuperscript{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.\textsuperscript{20}

Renal tubular function remained stable in this study, as indicated by serum and urine $\beta_2$-microglobulin concentra-
tions. The β2-microglobulin is a low molecular weight protein produced predominantly by lymphocytes. 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. 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.

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. 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.

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.

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