Extrahepatic Metabolism of Sevoflurane in Children Undergoing Orthotopic Liver Transplantation

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Background: **Sevoflurane is metabolized by cytochrome P450 and produces inorganic fluoride. The anhepatic phase of liver transplantation provides a useful tool to study the extrahepatic metabolism of drugs. The authors therefore studied the extrahepatic metabolism of sevoflurane by measuring the fluoride production in children receiving sevoflurane solely during the anhepatic phase of orthotopic liver transplantation.**

Methods: **Children with end-stage liver disease undergoing orthotopic liver transplantation were studied. Anesthesia was provided with isoflurane, sufentanil, and pancuronium. In one group, isoflurane was replaced by sevoflurane as soon as the liver was removed from the patient and maintained until reperfusion of the new liver. Arterial blood samples were drawn at induction, before removal of the liver, 15 min and 30 min after the beginning of the anhepatic phase, at the unclamping of the new liver, and finally 60 and 120 min after the unclamping. Plasma fluoride concentrations were determined by ion-selective electrode.**

Results: **No differences between the two groups (n = 10) regarding age, weight, duration of the anhepatic phase, or basal level of inorganic fluoride were found. The fluoride concentration increased significantly as soon as sevoflurane was introduced; it remained stable in the group receiving isoflurane. The peak fluoride concentration was also significantly higher in the first group (mean ± SD: 5.5 ± 0.8 μM (sevoflurane group) *versus* 1.4 ± 0.5 μM (isoflurane group) $P < 0.05$).**

Conclusions: **These results demonstrate the existence of an extrahepatic metabolism of sevoflurane at least in children with end-stage liver disease. (Key words: Biotransformation; halogenated agents; inhalation.)**

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THE metabolism of sevoflurane by cytochrome P450 2E1 has been extensively studied: It produces equimolar amounts of inorganic fluoride ions (F$^-$) and hexafluoroisopropanol.[1] The formation of F$^-$ may thus be considered a marker of sevoflurane metabolism.[2] This metabolism is supposed to occur mainly in the liver.[1] However, halothane, enflurane and methoxyflurane are also metabolized extrahepatically, mostly by the kidneys and the lungs, in addition to their predominant liver biotransformation.[3] The anhepatic phase of liver transplantation provides a useful tool to study the extrahepatic metabolism of drugs.[4,5] The present study investigates the possible extrahepatic metabolism of sevoflurane by measuring the F$^-$ production during the anhepatic phase in children undergoing orthotopic liver transplantation.

Materials and Methods

After approval of the local human ethics committee and informed consent of their parents, 26 children undergoing liver transplantation for biliary atresia were included in the study. They were randomly allocated to either group S or group I, receiving respectively sevoflurane or isoflurane during the anhepatic phase of orthotopic liver transplantation. Anesthesia was induced in both groups with halothane and 100% oxygen using a modified Ayre's T piece until tracheal intubation was performed. Controlled ventilation with a ventilator (Cicero, Dräger, Lubeck, Germany) using a circle circuit breathing system with soda lime and a fresh gas flow of 2 l/min. Minute volume was adjusted to obtain an arterial carbon dioxide concentration around 37 mmHg. Anesthesia was maintained with isoflurane in air/oxygen in both groups. End-tidal halogen concentrations were monitored continuously using a Datex (Helsinki, Finland) AS3 monitor. Analgesia was provided by continuous sufentanil infusion (0.3 μg · kg^{-1} · h^{-1}) and muscle relaxation by pancuronium. Perioperative antibiotherapy consisted of ampicillin (25 mg/kg) and ceftazi-

Table 1. Demographic Data of Patients and Fluoride Levels

	Group I	Group S	P
Age (mo)	36 ± 9	44 ± 20	0.5
Weight (kg)	13 ± 2	14 ± 3	0.4
Duration of anhepatic phase (min)	66 ± 6	60 ± 6	0.5
Basal F$^-$ (μM)	1.2 ± 0.5	0.9 ± 0.3	0.6
Peak F$^-$ (μM)	1.2 ± 0.5	5.5 ± 0.8	0.01

Values are mean ± SD.

Basal F$^-$ = basal fluoride concentration; Peak F$^-$ = maximum fluoride concentration.

dim (30 mg/kg); tranxenamic acid (20 mg/kg) was given to prevent perioperative fibrinolysis. Dopamine (2 μg · kg^{-1} · h^{-1}) and mannitol (60 mg/kg) were also infused. In group S, isoflurane was discontinued immediately after removal of the native liver, thus at the start of the anhepatic phase, and replaced by 2% end-tidal sevoflurane until just before unclamping of the vessels of the newly transplanted liver, when isoflurane was reintroduced.

Intravenous dextrose (5%) or saline was administered to provide maintenance fluid. Human albumin solution or bank blood was administered according to the fluid deficit or blood losses.

Arterial blood samples (2 ml) were collected in plastic heparinized syringes after induction of anesthesia (T0), immediately after removal of the native liver (T1), 15 min (T2) and 30 min (T2′) later, just before the unclamping of the newly transplanted liver (T3), and finally 60 (T4) and 120 min (T5) after reperfusion of the new liver. Blood was centrifuged and plasma was kept frozen at $-20°$C until determination of plasma F$^-$ concentrations with an ion-specific electrode (Orion Research, Cambridge, MA). A standard curve ranging from 1 μM to 50 μM was prepared each morning before the measurements by adding incremental doses of sodium fluoride to a Tris solution. The limit of detection was 0.5 μM, and the coefficient of variation of the assay at this concentration was approximately 6%.

Statistical Analysis

Statistical differences were detected with unpaired or paired *t* test; a *P* value < 0.05 was considered statistically significant. Results are given as mean ± SD.

Results

Patients requiring blood transfusion before or during the anhepatic phase were excluded from the analysis to avoid any contamination by exogenous fluoride. The demographic data of the remaining patients in group I (n = 10) and group S (n = 10) were comparable (table 1). As preoperative medications, all children received supplemental vitaminotherapy (A, D, E, K); three children of the I group and four of the S group received spironolactone. Basal F$^-$ concentrations were similar in both group and no significant changes were observed during the dissection of the native liver. Significant rises in F$^-$ concentrations were observed only in group S during the anhepatic phase; they remained stable in group I. The F$^-$ concentrations between S group and I group were significantly higher during the sevoflurane administration and remained different after reperfusion of the liver (fig. 1).

Discussion

This study demonstrates *in vivo* the existence of an extrahepatic metabolism of sevoflurane: This is shown by the increase of F$^-$ concentrations in a group of children receiving sevoflurane exclusively during the anhepatic phase of orthotopic liver transplantation. A group of comparable children receiving isoflurane throughout the whole procedure was used as control, because this agent is much less metabolized and pro-
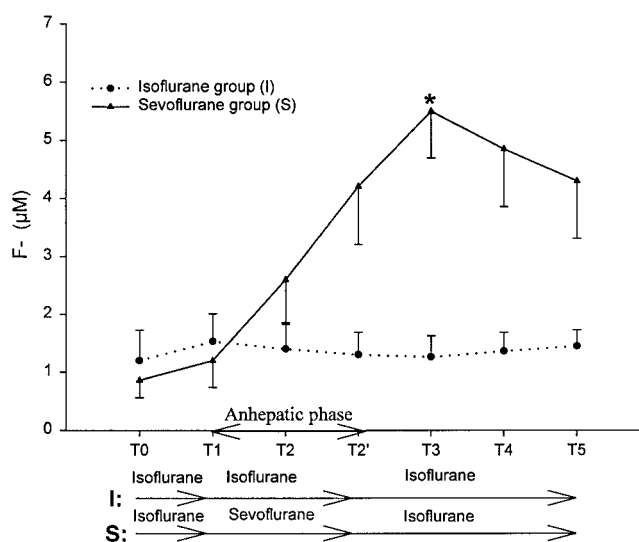

Fig. 1. Plasma fluoride (F$^-$) concentrations. T0 = basal level after induction of anesthesia, T1 = immediately after removal of the native liver; T2 = 15 min after T1; T2′ = 30 min after T1; T3 = just before the unclamping of the newly transplanted liver; T4 = 60 min after T3; T5 = 120 min after T3. *Significant comparing peak of sevoflurane group and isoflurane group. Values are mean ± SD.

duces only small amounts of F^-.[6] However, the exact location of the sevoflurane biotransformation is not yet determined. The possible biotransformation of halogenated agents by organs other than the liver has been suggested previously for halothane, enflurane, and for methoxyflurane.[3,7] However, these studies were performed on rabbit tissues, and species differences in the repartition of the CYP450 isoforms have also been demonstrated, which induces difficulties in extrapolating *in vitro* animal results to humans. On the other hand, the extrahepatic concentration of these different P450 isoforms varies according to the organ studied.[8] It appears therefore important to know the CYP450 isoform involved in the metabolism of the anesthetic agent studied. Some of them, such as enflurane, the oxidation of halothane,[9] and sevoflurane, are almost exclusively metabolized by the 2E1 isoform, whereas methoxyflurane and the reductive pathway of the halothane depend on others isoforms such as 1A2, the 2C9/10 and 2D6 isoforms,[10] or 2A6 and 3A4.[11] Because enflurane has thus a metabolic pathway similar to that of sevoflurane, it appears to be the sole agent able to provide information on the extrahepatic metabolism. Blitt *et al.*[3] have shown that enflurane is poorly metabolized by the lungs and the kidneys.

Studying the metabolism of sevoflurane and methoxyflurane by human kidney and liver microsomes, Kharash *et al.*[12] have shown that sevoflurane is almost exclusively biotransformed by the liver microsomes, as opposed to methoxyflurane, which is also metabolized by kidney microsomes. This is consistent with the fact that CYP450 2E1 is scarcely expressed in human kidney. These authors suggested that this local metabolism could be one of the factors involved in the more important renal toxicity of methoxyflurane when compared to sevoflurane.

Although the CYP450 2E1 concentration is highest in the liver, this cytochrome P450 isoenzyme has also been found in organs such as the gut and the lungs[13] and even in the lymphocytes.[14] These locations may therefore be the additional organs of extrahepatic metabolism of sevoflurane shown in our study. The presence of CYP450 2E1 in various tissue of the lung has been described, but the relative concentration seems very low in normal condition.[15] The lung microsomes are also three to four times less active than microsomes from the lung.[16] In two older children in whom a pulmonary artery catheter had been inserted, no difference between F^- concentrations measured simultaneously in the pulmonary arterial blood and the radial arterial blood at the

end of the anhepatic phase was observed. The presence of a lower level of F^- in the pulmonary artery than in the systemic arterial blood could have demonstrated a pulmonary metabolism.[17] However, more cases are required to definitively elucidate the importance of the lungs as one of the sites of this extrahepatic breakdown. On the other hand, even if studied individually each organ appears less potent compared with the liver; the addition of the different extrahepatic locations in which CYP450 has been found represents a large metabolic capacity, accounting probably for the importance of the extrahepatic metabolism of sevoflurane that we have shown in this study.

It is impossible with this study to quantify the relative efficiency of the extrahepatic biotransformation as compared with the liver metabolism; however, Levine *et al.*[18] found a linear correlation between the amount of sevoflurane delivered to the patient and the amount of fluoride produced. They found a peak plasma F^- ranging between 7 and 28 μM after a mean of 2.7 minimum alveolar concentrations per hour. The fluoride concentration in children receiving only one minimum alveolar concentration per hour of sevoflurane was around 11 μM; this concentration was 1 μM in the control group receiving halothane; because this drug releases only minor quantities of inorganic fluorides, we assume that this was the basal level in both groups. In our study, the children only received 2% end-tidal sevoflurane for an average of 58 min, which is less than one minimum alveolar concentration per hour. Despite this lower amount of inhaled sevoflurane, our patients increased their F^- concentration from a basal level of 0.9 to a peak concentration of 4.5 μM. This represents about one third of the increase observed in Levine's study, showing that the extrahepatic metabolism accounts for one third of the whole seroflurane metabolism. We must, however, stress that our study has been performed in children suffering from long-term liver cirrhosis, which could lead to a compensatory increased activity of the cytochromes located extrahepatically; further studies are required to confirm this hypothesis.

In conclusion, we have shown that sevoflurane undergoes extrahepatic metabolism. However, more studies measuring the F^- gradient existing between the inflow and the outflow of blood perfusing the kidneys, lungs, or gut are required to determine the relative contribution of these organs to this extrahepatic biotransformation.

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