Serum Fluoride Concentration after Sevoflurane Anesthesia in Ethanol Treated Rats: Special Reference to Cytochrome P-450 in the Liver

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The relationship between serum concentration of inorganic fluoride (F^-) and cytochrome P-450 content after sevoflurane anesthesia was investigated in ethanol treated rats. Twenty male Wistar rats were randomly divided into 2 isocaloric diet groups of 10 rats each: one group receiving a standard diet and the other an ethanol diet. After 28 days on the diets the animals were administered 2.5% sevoflurane for 2 hr with 30% oxygen and 70% nitrous oxide. Cytochrome P-450 and cytochrome b_5 were induced by the ethanol diet. In the ethanol diet group serum concentration of F^- was significantly higher than that of the standard diet group after sevoflurane anesthesia. These results suggest that cytochrome P-450 and b_5, which were induced by ethanol, enhanced sevoflurane defluorination. (Key words: sevoflurane, ethanol, inorganic fluoride, cytochrome P-450, cytochrome b_5)

(Masaki E, Kondou T, Hirakawa J, et al.: Serum fluoride concentration after sevoflurane anesthesia in ethanol treated rats: special reference to cytochrome P-450 in the liver. J Anesth 6: 426–432, 1992)

Sevoflurane, is an ether containing seven fluoride atoms. Although the complete pathway of metabolism of sevoflurane is not known, it has been shown that inorganic fluoride (F^-) was released when sevoflurane is metabolized in the body[1]. There have been several reports about the serum concentration of F^- in studies of normal and barbiturate enzyme induced livers[2,3]. These studies demonstrated that serum concentration of F^- during and after sevoflurane anesthesia does not reach levels known to cause renal dysfunction. However, in these studies, cytochrome P-450 concentration was not measured, and it is known the hepatic cytochrome P-450 isozymes, which are known to oxidize halogenated hydrocarbons, are not well induced by phenobarbital. On the other hand, in related work, it has been reported that ethanol induces cytochrome P-450 isozymes[4,5]. Furthermore, the cytochrome P-450 isozyme induced by ethanol are different from those induced by barbiturate[6,7] and are closely contributed to the oxidation of halogenated hydrocarbons. While it has been reported that ethanol treatment significantly enhances defluorination of sevoflurane[8], these studies have been done *in vitro* with no determina-

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tion made of *in vivo* level of serum F^- in ethanol treated animals.

Therefore we investigated the relationship between cytochrome P-450 and b_5 contents and serum concentration of F^- in ethanol treated rats anesthetized with sevoflurane. Aim of this study was directed at the qualitative assessment of ethanol pretreatment effects on metabolism of sevoflurane. Results may be directly applicable to clinical cases of anesthesia with people who drink alcohol chronically.

Materials and Methods

Wistar rats (200–250 gms) were divided into two groups of 10 animals each and fed either a liquid standard diet or a liquid ethanol diet in which ethanol replaced the carbohydrate isocalorically. Preparation of the liquid diet generally followed the method of DeCarli and Liber[9], in which the basic diet provided 5% of total calories as fat, 8% as protein, 87% as carbohydrate reflecting the nutritional background of patients with alcoholic liver damage[10]. For the ethanol diet, carbohydrate was isocalorically replaced by ethanol to the extent of 42% of the total calories. Both of these diets contained adequate vitamins and minerals. The two groups of rats were pair fed isocalorically for 4 weeks and at this time all rats were immediately fasted for 12 hr before exposure to the anesthetic.

Sevoflurane was administered from Sevotec 3 in a 10 liter plastic chamber at 2.5% with 30% oxygen and 70% nitrous oxide. The average gas flow through the chamber was 3 liter·min^{-1}. Expiratory gas was monitored with a PERKIN-ELMER 1100 Medical Gas Analyzer. The animals were anesthetized for 2 hrs and then were withdrawn their blood from the inferior vena cava for determination of serum F^- and the level of plasma liver enzymes (GOT, GPT, LDH, γ-GTP). Serum F^- was measured using Orion research ionanalyzer and the quantity of plasma liver enzymes were measured by standard procedures.

Table 1. Effect of ethanol treatment of rats on body weight, liver weight and protein contents

	standard	ethanol
Final body weight (g)	186.3 ± 6.0	205.2 ± 6.0
Liver weight (g)	6.9 ± 0.4	7.5 ± 0.3
Liver weight/ 100g body weight	3.9 ± 0.2	3.7 ± 0.1
Hepatic microsomal protein (mg·g^{-1} liver)	12.9 ± 1.0	15.9 ± 1.5

All values are expressed as mean ± SE n=10

The liver was then excised and a small sample taken for histological examination and remainder immediately placed in an ice-cold 100 mM potassium phosphate buffer containing 10 mM EDTA (pH=7.4) and homogenized. The homogenate was centrifuged for 10 min at 12,000 × g and the supernatant was ultracentrifuged for 60 min at 105,000 × g. The resultant microsomal pellet was resuspended in 10 mM Tris-HCl buffer containing 0.15 M KCl and 1 mM EDTA (pH=7.4) and recentrifuged as before. The washed microsome pellet was resuspended in 100 mM potassium phosphate buffer containing 10 mM EDTA (pH=7.4). Protein concentration was determined by a Bradford method[11].

Cytochrome P-450 and cytochrome b_5 contents were measured by the method of Omura and Sato[12], using the extinction coefficients of 91 mM^{-1}·cm^{-1} and 185 mM^{-1}·cm^{-1} respectively.

The data were analyzed for statical significance by using Student's paired t-test.

Table 2. Effect of ethanol treatment of rats on the activity of plasma liver enzymes

		standard	ethanol
SGOT	(IU/L)	65.9 ± 12.6	86.9 ± 14.1
SGPT	(IU/L)	27.3 ± 4.3	38.2 ± 6.3
LDH	(IU/L)	390.2 ± 153.4	338.7 ± 83.5
γ-GTP	(IU/L)	low	low

All values are expressed as mean ± SE n=5

Results

Body weight, liver weight and hepatic microsomal protein content tended to increase slightly in the ethanol diet group (table 1).

There were no significant changes between the two groups in histological findings by light microscopy (data not shown). However, in histological findings by electron microscopy, a proliferation of the smooth endoplasmic reticulum and crystalline inclusion in mitochondria, which are characteristic of chronic ethanol administration, were evident in the ethanol treated group (fig. 1).

There were no significant differences between both groups in the activity of plasma liver enzymes (table 2).

As shown in table 3, cytochrome P-450 and b_5 were induced by the treatment of ethanol diet. In the ethanol diet group, serum concentration of F^- was significantly higher than that of the standard diet group.

Table 3. Effect of ethanol treatment of rats on contents of hepatic cytochrome P-450, b_5 and serum F^-

	standard	ethanol
cytochrome P-450		
n moles·mg^{-1} protein	1.16 ± 0.12	1.46 ± 0.12
n moles·g^{-1} liver	14.7 ± 1.63	21.8 ± 0.73**
cytochrome b_5		
n moles·mg^{-1} protein	0.48 ± 0.04	0.55 ± 0.04
n moles·g^{-1} liver	6.09 ± 0.46	8.27 ± 0.35**
serum F^- (μM)	4.21 ± 0.37	9.99 ± 0.58***

All values are expressed mean ± SE n=10
**$P < 0.01$, ***$P < 0.001$ vs standard

Discussion

One of the features of this study is the composition of the diet. The difficulty of the enzyme-induced effects of chronic ethanol consumption is alteration in nutritional state. Therfore, we changed the composition of diet to mimic as closely as possible the nutrient intake of patients with alcoholic liver damage.

Results of this study indicate that cytochrome P-450 and b_5, which were increased by the chronic ethanol administration, enhanced sevoflurane defluorination and raised the serum concentration of F^-. Previous studies suggest that serum concentration of F^- does not increase in phenobarbital treated rats after sevoflurane anesthesia[3]. Two major explanations for the difference of sevoflurane metabolism between ethanol and phenobarbital treatment should be considered. First, the study suggests that cytochrome P-450 isozymes induced by ethanol are different from those induced by phenobarbital[6,7]. In morphologic observations, drugs such as ethanol, phenobarbital, isoniazid and pheytoin, increased microsomal protein content and the activity of hepatic microsomal enzymes, both in smooth and rough microsomal fractions with a predominance of effect in the smooth membranes[13,14] and this agreed with the proliferation of the smooth endoplasmic reticulum[15]. This finding is consistent with our result as shown in figure 1. However, in contrast to ethanol treatment, it was reported that phenobarbital produced no appreciable morphological changes in the

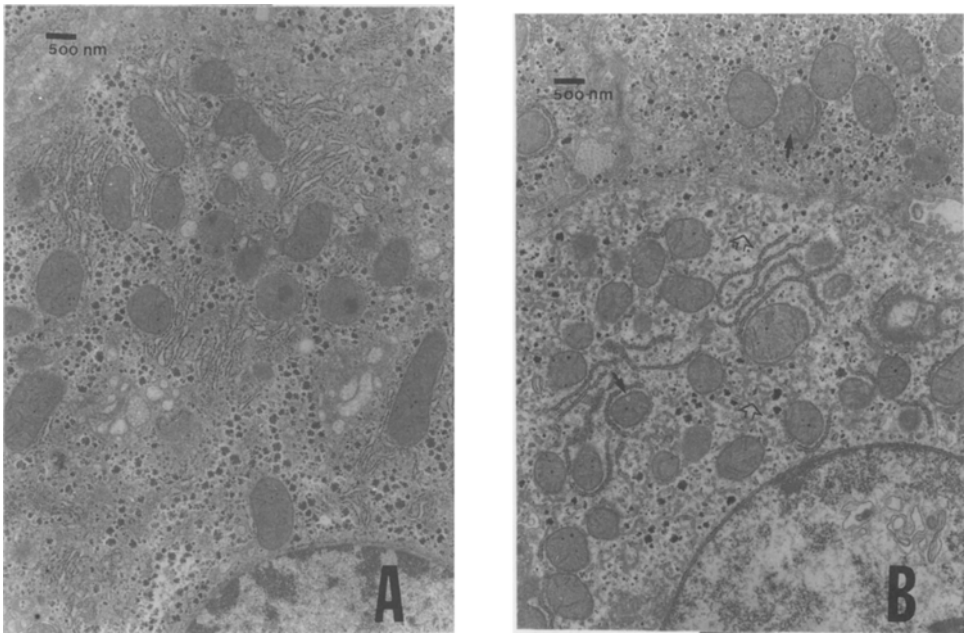

Fig. 1. Histological findings by electron microscopy.
A; standard diet group, B; ethanol diet group. A proliferation of the smooth endoplasmic reticulum (⇒) and crystalline inclusion in mitochondria (➡) are evident in ethanol diet group. Both of these things are characteristic of chronic ethanol administration.

rough endoplasmic reticulum[16]. Drugs-induced proliferation of smooth endoplamic reticulum is generally accompanied by an increase in cytochrome P-450 and hepatic microsomal drug metabolizing enzymes[13]. These different type of cytochrome P-450 isozymes were reported to metabolize anesthetics differently. The defluorination of enflurane was more stimulated by ethanol treatment than by phenobarbital treatment, while methoxyflurane was defluorinated to a greater extent by phenobarbital than by ethanol[17]. Comparison of defluorination of enflurane and methoxyflurane suggested the possibility that the ethanol inducible cytochrome P-450 enhanced the enzymatic activity of the dehalogenation of terminal carbon of the ether[17]. Another report demonstrated that enflurane was defluonated and serum concentration of F^- was significantly increased by ethanol treatment *in vivo*[18], while conventional phenobarbital treatment produced little or no enhancement of enflurane metabolism[19]. Sevoflurane, like enflurane, was defluorinated in both phenobarbital and imidazole induced hepatic microsomal preparations and rate of defluorination was much higher in imidazole induced hepatic microsomal preparations[20]. Imidazole was considered to induce the same cytochrome P-450 isozymes which are inducible by ethanol[21]. These findings indicate that the different forms of cytochrome P-450 have different substrate specificities among the anesthetics and the ethanol induced cytochrome P-450 isozymes oxidize the terminal carbon of the ether of sevoflurane. Secondly, in the present study, ethanol induced not only cytochrome P-450 but cytochrome b_5. Cytochrome b_5 has been reported to be involved in the microsomal mixed function ox-

idase reaction by providing a second electron to cytochrome P-450[22]. Methoxyflurane, which yields F^- as a product of its metabolism and produces renal toxicity as a consequence of this metabolism, interacts with the substrate binding site of cytochrome P-450 to induce requirements of cytochrome b_5[23]. As a result, the second electron for cytochrome P-450-catalyzed oxidation is donated exclusively by cytochrome b_5 for methoxyflurane oxidation[23]. Another study reported that purified ethanol-inducible cytochrome P-450 metabolized volatile anesthetics including sevoflurane at a rate stimulated four-fold by cytochrome b_5[20]. On the other hand, phenobarbital treatment was reported to cause a elevation of cytochrome P-450 content but no change, or, only small elevation of cytochrome b_5 content in the microsome[24]. It is possible that the defluorination of sevoflurane by the ethanol induced cytochrome P-450 was enhanced by the ethanol induced cytochrome b_5 and ther by contributes to the significant increase in serum concentration of F^- in ethanol treated rats.

Although, this study indicated that ethanol-inducible cytochrome P-450 and b_5 enhanced sevoflurane defluorination and increased serum concentration of F^-, it should also be noted that the isozyme of P-450 induced by ethanol is increased only transiently, with the peak level occurring during ethanol treatment and decreasing to the baseline levels within 24 hours following removal of the ethanol[17,25]. Thus the level of F^- reported here are maximal since they were measured within 24 hours of ethanol treatment.

While serum concentration of F^- increased in the ethanol diet group, the activity of plasma liver enzymes were not affected by treatment of ethanol (there were no significant differences between our results on the activity of plasma liver enzymes and normal data[26]). This result indicates that sevoflurane did not cause liver damage in ethanol treated rats.

In conclusion, this study demonstrated that the metabolism of sevoflurane was associated with ethanol induced cytochrome P-450 and b_5 and serum concentration of F^- was significantly increase by ethanol treatment although the peak level of F^- were well below the concentration threshold for nephrotoxicity.

Acknowledgments: This study was supported in part by a Research Grant from the Ministry of Education, Science and Culture of Japan. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, Nevada, October, 1990.

(Received Oct. 1, 1991, accepted for publication Feb. 4, 1992)

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