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Liver Function and Anesthetic Metabolism in Rats with Chronic Renal Impairment

Susan A. Rice, Ph.D., Timothy S. Sievenpiper, M.D.,† Richard I. Mazze, M.D.‡*

Patients and rats with chronic renal insufficiency (CRI) anesthetized with enflurane do not have significantly greater increases in postoperative serum inorganic fluoride levels when compared with subjects with normal renal function. The authors chose to investigate whether this observation is due to decreased anesthetic metabolism, secondary to the renal disease. Thus, male Fischer 344 rats with surgically induced CRI were studied to determine the effect of severe renal impairment: first, on *in vivo* hepatic function as measured by a serum liver enzyme profile, and second, on *in vitro* hepatic metabolism as indicated by microsomal anesthetic defluorination rates and cytochrome P-450 levels. Rats were operated on in two stages, 1 week apart, and assigned to one of three groups. Group 1 rats had a capsule stripping of each kidney. Group 2 rats had a capsule stripping of one kidney and then a nephrectomy of the other. Group 3 rats had the upper and lower poles of one kidney excised and then a nephrectomy of the other. There was no change in renal function in rats from Group 1 and 2. Chronic renal insufficiency in Group 3 rats was manifested by threefold elevations in serum creatinine and urea nitrogen levels and reciprocal decreases in clearances. After 89–98 days, blood was obtained for a serum liver enzyme profile and rats were killed for determination of *in vitro* hepatic metabolism. There were no changes suggestive of hepatic damage. Although there was an approximate 25% decrease in hepatic cytochrome P-450 content in the Group 3 rats, there was no evidence of altered drug metabolism as indicated by the rates of defluorination of methoxyflurane, enflurane, isoflurane, or sevoflurane. Lack of unusually high fluoride levels and subsequent nephrotoxicity in subjects with CRI anesthetized with enflurane is apparently not due to decreased hepatic defluorination of the anesthetic. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane; methoxyflurane; sevoflurane. Biotransformation. Kidney: failure. Liver: function. Metabolism. Toxicity.)

METHOXYFLURANE ($CHCl_2CF_2$-O-CH_3), a difluorinated methylethyl ether, causes polyuric renal insufficiency in surgical patients and in Fischer 344 rats as a consequence of its biotransformation to inorganic fluoride (F^-).[1,2] The lesion is dose related[3,4] and its development more likely in enzyme-induced subjects.[5–7] Enzyme inhibition with SKF-525A decreases defluorination of methoxyflurane, which prevents the renal lesion.[5] Because of its nephrotoxic potential, methoxyflurane has, in recent years, all but dropped from clinical anesthesia practice. Enflurane ($CHClFCF_2$-O-CF_2H), a methylethyl ether subsequently developed, also is defluorinated but to only about one-third the extent of methoxyflurane.[7–9] It was postulated that its administration to patients with renal insufficiency would result in persistently high serum F^- levels. Fluoride would accumulate as a result of decreased excretion secondary to reduced glomerular filtration rate and produce a renal lesion. Studies of patients and of rats, however, have not supported this prediction. The study of anephric surgical patients by Carter *et al.*,[10] that of patients with mild to moderate chronic renal insufficiency (CRI) by Mazze *et al.*,[11] and the study of Fischer 344 rats by Sievenpiper *et al.*[12] demonstrated that serum F^- levels 1 day following enflurane anesthesia increased no more in subjects with CRI than in those with normal renal function. This observation is somewhat surprising, considering that normally 40–50% of a F^- dose is excreted by the kidney within 24 h, while the rest primarily is sequestered into bone.[13,14]

There are several explanations for this observation. We chose to investigate one hypothesis: that the unexpectedly low serum F^- levels in human and animal subjects with CRI were due to decreased hepatic anesthetic metabolism. Thus, the present study was designed to determine the effect of severe CRI: first, on *in vivo* hepatic function as measured by a serum liver enzyme profile, and second, on *in vitro* hepatic metabolism as indicated by microsomal anesthetic defluorination rates and cytochrome P-450 levels.

Materials and Methods

Male Fischer 344 rats,§ 11 months old at the start of the study, were housed in cages on corn-cob bedding¶ for 2 weeks prior to operation. Room temperature (21 ± 1° C) and light (0600 to 1900 h) were controlled, and rats were fed standard laboratory chow** and tap water *ad libitum* throughout the study except during anesthetic

* Assistant Professor of Pharmacology and Toxicology in Anesthesia (SUSM) and Pharmacologist (PAVAMC).

† Assistant Professor of Anesthesia (SUNY).

‡ Professor of Anesthesia (SUSM) and Chief, Anesthesiology Service (PAVAMC).

Received from the Department of Anesthesia, Stanford University School of Medicine (SUSM), Stanford, California, the Department of Anesthesia, State University of New York (SUNY), Buffalo, New York, and Anesthesiology Service, Veterans Administration Medical Center (PAVAMC), Palo Alto, California. Accepted for publication November 2, 1984. Supported in part by NIH Grant GM22746 and the Veterans Administration, Palo Alto, California.

Address reprint requests to Dr. Rice: Anesthesiology Service (112A), Veterans Administration Medical Center, 3801 Miranda Avenue, Palo Alto, California 94304.

§ Microbiological Associates, Walkerville, Maryland 21793.

¶ Bed-O' Cobs, Anderson's Cob Division, Maumee, Ohio 43537.

** Wayne Lab-Blox, Allied Mills Inc., Chicago, Illinois 60951.

TABLE 1. Body and Liver Weights and Creatinine Clearance of Rats with CRI (Mean ± SD)

Group	Surgery	n	Body Weight (g)		Creatinine Clearance* ml·min^{-1}·100 g body weight^{-1}		Liver Weight (g)	Liver Weight as Per Cent of Body Weight
			Prior to Operation	At Death	Prior to Operation	Day Prior to Death		
1	Sham	7	383 ± 16	396 ± 26	0.42 ± 0.15	0.58 ± 0.21	12.9 ± 1.2	3.3 ± 0.2
2	Unilateral	5	376 ± 22	383 ± 20	0.37 ± 0.07	0.49 ± 0.01	12.3 ± 1.4	3.2 ± 0.2
3	Bilateral	15	384 ± 11	308 ± 47†	0.38 ± 0.05	0.11 ± 0.07[b]	11.1 ± 2.2	3.6 ± 0.3[b]

* Modified from Sievenpiper *et al.*[1]

† Significantly different from Groups 1 and 2 ($P < 0.05$).

exposure. Replicate experiments were performed on two separate occasions. Chronic renal insufficiency was produced surgically by excision of kidney tissue on two consecutive weeks. Group 1, the control group, had a capsule stripping of the left kidney, followed by a capsule stripping of the right kidney 1 week later; Group 2 had a capsule stripping of the left kidney the first week followed by a right nephrectomy on the second week; Group 3 had an excision of the upper and lower poles of the left kidney followed by a right nephrectomy 1 week later. The groups are referred to in the text as the sham, unilateral, and bilateral nephrectomy groups. There was no change in renal function in the sham and unilateral nephrectomy groups. Chronic renal insufficiency developed after the second operation in the bilateral nephrectomy groups and was manifested by threefold elevation in serum creatinine and urea nitrogen levels and reciprocal decreases in clearances (table 1).

Representative creatinine clearances are presented in table 1. As part of another experiment, rats in Groups 1 and 2, and 8 of the 15 rats in Group 3, were exposed on two occasions (days 42 and 62 after the second operation) to 2% enflurane; the remaining seven rats in Group 3 received 1% halothane. There were no lasting effects from these treatments, as has been reported previously.[12]

At the termination of the experiment, approximately 3 months following the operation (89–98 days), rats were killed by decapitation. A blood sample was taken for a liver serum profile (Technicon SMA-12®; Technicon Instruments Corporation, Tarrytown, New York 10591), which included: aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), alkaline phosphatase, cholesterol, uric acid, inorganic phosphate, ionized calcium, globulin, total protein, and total bilirubin. Livers then were removed, rinsed in 0.9% NaCl, blotted dry, and weighed. Tissues were sliced 3–4 mm thick, and the paraffin-embedded slices were fixed in 10% buffered formalin. Paraffin sections were cut at 4–6 μm and were stained with hematoxylin and eosin. Light microscopic examination of coded slices was performed with the experimental group unknown to the examiner.

The remaining liver was perfused with cold 1.15% KCl, and microsomes were prepared as reported previously.[9] Microsomal protein and cytochrome P-450 contents were determined.[15,16] The microsomal defluorination rates for four anesthetics (methoxyflurane [MOF], enflurane [ENF], isoflurane [ISO], and sevoflurane [SEVO]) were determined as previously reported.[9] Inorganic fluoride (F^-) released from anesthetic metabolism during a 15-min incubation at 37° C was measured by an Orion ion-specific electrode and a model 801 Orion Ion-analyzer.[17]

Data were assessed by one-way analysis of variance. Where indicated, differences among groups were determined using the Neuman–Keuls *post hoc* test. A probability of $P < 0.05$ was considered significant.

Results

At death, the sham and unilateral nephrectomy groups had equivalent body weights (table 1). Rats with severe CRI had significantly decreased body weights, compared with their own preoperative weight and to the sham and unilateral groups at death. Liver weights among groups were not significantly different (table 1). However, as a result of decreased body weight, liver to body weight

TABLE 2. Serum Liver Function Studies of Rats with CRI (Mean ± SD)

Group	Surgery	N	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase (U/L)	Cholesterol (mg/dl)
1	Sham	7	279 ± 31	142 ± 59	244 ± 33	67 ± 4
2	Unilateral	5	261 ± 43	111 ± 26	253 ± 60	74 ± 10
3	Bilateral	15	198 ± 87*	53 ± 27†	195 ± 66	146 ± 34†

* Significantly different from Group 1 ($P < 0.05$).

† Significantly different from Groups 1 and 2 ($P < 0.05$).

TABLE 3. Serum Liver Function Studies of Rats with CRI (Mean ± SD)

Group	Surgery	N	Uric Acid (mg/dl)	Inorganic Phosphate (mg/dl)	Ionized Calcium (mg/dl)	Globulin (gm/dl)	Total Protein (gm/dl)	Total Bilirubin (mg/dl)
1	Sham	7	1.4 ± 0.2	5.9 ± 0.4	11.2 ± 2.1	2.8 ± 0.2	6.8 ± 0.2	0.17 ± 0.05
2	Unilateral	5	1.3 ± 0.2	5.7 ± 0.4	10.6 ± 0.3	2.8 ± 0.2	6.4 ± 0.4	0.16 ± 0.05
3	Bilateral	15	1.7 ± 0.6	10.3 ± 5.6	10.2 ± 2.5	2.7 ± 0.4	5.7 ± 0.4*	0.24 ± 0.10

* Significantly different from Groups 1 and 2 ($P < 0.05$).

ratios were increased in the bilateral nephrectomy group (Group 3) as compared with Groups 1 and 2 ($P > 0.05$). Light microscopic examination of liver tissue did not reveal any morphologic differences among groups. Similarly, the serum liver profile showed no evidence of hepatic injury or dysfunction (tables 2 and 3), although some measurements showed significant differences among groups. SGPT and total protein were decreased significantly ($P < 0.05$) in the bilateral nephrectomy group, while SGOT and total bilirubin were no different than control. Serum cholesterol was increased significantly ($P < 0.05$) in the bilateral nephrectomy group.

Table 4 summarizes the effect of CRI on cytochrome P-450 levels and rates anesthetic defluorination in microsomes prepared from the livers of these rats. There was a significant decrease in cytochrome P-450 content in the bilateral nephrectomy group rats (Group 3) compared with the sham and unilateral nephrectomy groups ($P < 0.05$). Among the anesthetics, methoxyflurane was defluorinated to the greatest extent, while isoflurane was defluorinated to the least. Renal impairment did not affect the extent of defluorination of any of the anesthetics either expressed in terms of milligrams of microsomal protein (table 4) or in nanomoles of cytochrome P-450.

Discussion

Previous studies have shown that serum F^- levels in humans and rats with CRI receiving enflurane anesthesia unusually are not increased. In a study of anephric patients anesthetized with enflurane, Carter *et al.*[10] reported no differences in peak serum F^- levels or mean levels 24 h postoperatively compared with a normal control group. Patients with severe renal insufficiency (mean creatinine clearance < 5 ml/min) had F^- levels only 6 μM greater than control patients with normal renal function after 24 h anesthesia. In another study of patients with mild to moderate CRI (24 h creatinine clearance, 31 ± 5 ml/min), Mazze *et al.*[11] reported that serum F^- peaked at 19.0 μM 4 h after operation then decreased to 16.8 μM at 24 h, 11.0 μM at 48 h, and 7.0 μM at 72 h. These values are similar to those seen in patients with normal renal function anesthetized with enflurane. These results were unexpected, since approximately half of an administered dose of NaF[13,14] and presumably half of the F^- resulting from metabolism of the fluorinated anesthetics[18] usually is excreted by the kidney. Relatively normal F^- levels could be explained by decreased anesthetic metabolism in CRI as a result of concurrent hepatic impairment. However, in the present study, using a chronic uremia rat model, all the changes we observed were consistent with renal failure unaccompanied by hepatic failure. Decreased serum protein levels in Group 3 rats were due to decreased albumin levels as suggested by unaltered globulin levels. Considering the lack of changes in aspartate aminotransferase (SGOT) and bilirubin concentrations, the decreased protein levels are consistent with renal protein loss rather than with decreased production due to hepatic failure. The decreased alanine aminotransferase (SGPT) levels are not consistent with acute liver injury, which would be associated with increased transaminase levels.

Terminal hepatic failure could result in decreased levels such as those we observed in SGPT. However, had terminal hepatic injury been present, other indicators of abnormal liver function would have been evident. This was not the case. Finally, the elevated cholesterol levels observed in Group 3 rats are consistent with renal dysfunction, as may be seen, for example, in subjects with nephrotic syndrome.

TABLE 4. Cytochrome P-450 and Anesthetic Defluorinase Activities in Hepatic Microsomes of Rats with CRI (Mean ± SD)

Group	Surgery	N	Cytochrome P-450*	Methoxyflurane†	Enflurane†	Isoflurane†	Sevoflurane†
1	Sham	7	0.83 ± 0.22	17.3 ± 3.1	7.3 ± 2.6	2.1 ± 0.6†	9.1 ± 3.0
2	Unilateral	5	0.82 ± 0.23	17.0 ± 3.1	6.4 ± 2.3	3.3 ± 2.0	7.1 ± 1.2
3	Bilateral	15	0.60 ± 0.13§	18.5 ± 6.6	8.6 ± 3.9	4.0 ± 2.3	9.1 ± 4.2

* In nmol/mg protein.
† In nmol $F^- \cdot 5$ mg protein$^{-1} \cdot 15$ min^{-1}.
‡ N = 6.
§ Significantly different from Groups 1 and 2 ($P < 0.05$).

Volatile halogenated anesthetics are metabolized by the hepatic mixed function oxidase system, of which cytochrome P-450 is the terminal oxygenase. The activity of this system in the presence of uremia is incompletely understood. Rats rendered uremic by subtotal nephrectomy,[18,19] ligation of the ureters, or uranyl nitrate injection[20,21] have been studied in the acute phase, 2–10 days following production of the renal lesion. Hepatic cytochrome P-450 levels were decreased[19,20] or unchanged[21]; aminopyrine N-demethylase activity was decreased[19]; acetanilid hydroxylase activity was decreased[19]; and p-nitroanisole O-demethylase[19] and aniline hydroxylase activities[20] were unchanged. In the present study utilizing a chronic (89–98 days) uremia rat model, although hepatic cytochrome P-450 levels were approximately 25% less in rats with CRI than in control rats, microsomal mixed function oxidase defluorination rates for MOF, ENF, ISO, and SEVO were unaltered. These anesthetics were chosen because they are defluorinated to different extents, whether activity is measured *in vivo* or *in vitro,* and because they are metabolized to varying degrees following treatment with different enzyme-inducing and enzyme-inhibiting drugs. Thus, despite the somewhat lesser enzyme content in rats with CRI, hepatic defluorination of volatile fluorinated anesthetics is unimpaired.

If decreased hepatic metabolism is not responsible for the normal serum F^- levels seen in surgical patients and in animals with CRI following enflurane administration, what is the mechanism? We speculate that extrarenal redistribution, such as sequestration of F^- into bone, prevents significant increases in serum F^- levels in the presence of CRI and protects diseased kidneys from further damage. In support of this concept is the finding that in anuric patients, 80% of an injected dose of ^{18}F was taken up by bone in 1 h.[22] Also, in rats with CRI anesthetized with enflurane,[12] serum F^- levels decreased rapidly after peaking 4 h after anesthesia, despite minimal urinary F^- excretion. Redistribution of F^- into bone is the most likely explanation for these events.

In summary, in this animal model with CRI there were neither serious aberrations in liver function, nor were there alterations in defluorination rates of halogenated ether anesthetics. It is likely that normal serum F^- levels following enflurane anesthesia in patients with abnormal renal function are due to redistribution rather than decreased defluorination.

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