Acute Toxicity of a Nuclear Magnetic Resonance Cerebral Blood Flow Indicator in Cats

Craig A. Branch, PhD, James R. Ewing, MS, Susan C. Fagan, PharmD, David A. Goldberg, MD, and K.M.A. Welch, MD

We studied trifluoromethane as a potential gaseous indicator in nuclear magnetic resonance measurements of cerebral blood flow. We considered the effects of trifluoromethane on cerebral blood flow in 17 cats and on the electroencephalogram and electrocardiogram in nine cats and compared these with the effects of the more toxic compound chlorodifluoromethane in five cats. Inhaled at 60%, trifluoromethane had no effect on cerebral blood flow, the cerebral metabolic rate for oxygen, or oxyhemoglobin content. At 70%, trifluoromethane sensitized the cats' hearts to epinephrine, but to a much lesser degree than 40% chlorodifluoromethane, and produced only moderate changes in cerebral electrical activity as measured by the electroencephalogram.

We found trifluoromethane to be suitable for use in animals, but its toxicity needs to be studied further before it can be used in humans for the measurement of cerebral blood flow. (Stroke 1990;21:1172-1177)

Gaseous halocarbons have great potential in nuclear magnetic resonance (NMR) indicator-dilution measurements of cerebral blood flow (CBF).1-5 However, in animals acute exposure to halocarbons can alter pulmonary compliance and resistance, can depress myocardial contractility, arterial blood pressure, and coronary blood flow, and can sensitize the myocardium to epinephrine-induced arrhythmias.6-20 Extended exposure can cause pulmonary hemorrhage and congestion.21 A sparse literature indicates that these effects are apparently absent for trifluoromethane (FC-23).6-22 One report6 demonstrated no change in cardiac sensitivity to epinephrine during FC-23 inhalation, although small changes in sensitivity might not have been discerned because of the large doses of epinephrine administered. In another report22 no toxic symptoms were observed in dogs, although one author stated that during inhalation of FC-23 he experienced “definite analgesia and impairment of consciousness.”

Our initial studies of CBF measured with NMR in cats employed chlorodifluoromethane (CFC-22) because its high blood solubility,1 relatively low toxicity,17 and low cost were well suited to the development of NMR methodologies. With these methodologies proven, CFC-22 was discarded in favor of FC-23 because we found that 40% CFC-22 increased CBF by approximately 50%1. Despite an additional fluorine atom in FC-23, its signal-to-noise ratio in NMR experiments was poorer than that of CFC-22 since the former is roughly one third as soluble as the latter in blood.2 Thus, it was necessary to administer FC-23 at the highest possible concentration to attain bicompartimental curve fits to the clearance data so that relative weights of the cerebral compartments could be estimated.

Recently we2 reported multicompartmental NMR measurements of CBF using FC-23 as an indicator, along with the preliminary finding that FC-23 had no effect on CBF in nine cats. Because of the limited statistical significance of our results using so few animals, as well as because of recent reports to the contrary,4 we studied CBF and the cerebral metabolic rate for oxygen (CMRO2) in additional cats. We also studied the acute cardiac toxicity of FC-23 and its influence on the electroencephalogram (EEG) when administered at the concentration and duration required for NMR measurement of CBF. For comparison, we also evaluated the effects of CFC-22.
Materials and Methods

We studied the effect of 60% FC-23 on CBF in 17 cats using the Kety-Schmidt N2O clearance technique.23,24 Cats were anesthetized with 1.5 mg/kg i.v. xylazine and 15 mg/kg i.m. ketamine, tracheotomized, paralyzed with 0.08 mg/kg i.v. pancuronium bromide, and mechanically ventilated. A femoral artery and vein were cannulated for measurements of arterial blood pressure, blood gases, and N2O content and for the administration of fluids, respectively. The sagittal sinus was cannulated rostrally with a 24-gauge catheter for measurements of cerebral venous N2O and O2 contents.

Each measurement of CBF was preceded by the administration of 5 mg/kg i.v. thiopental sodium 5 minutes before the administration of 2 mg/kg i.v. ketamine. This combination produces a stable CBF approximately 80% of normal levels.1,22 Cats were ventilated with 30% O2+5% N2O+65% N2 for 1 hour before the measurement of control CBF by N2O clearance. The N2O was then replaced with N2, and the measurement of CBF began. The measurement of CBF during FC-23 inhalation was accomplished by replacing 60% of the N2 with FC-23 10 minutes before and during the N2O clearance period. Arterial and cerebral venous blood samples (0.3 ml aliquots) were taken before and after 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 12, 15, 30, and 45 minutes after N2O clearance into 1-ml plastic syringes. Each sample was immediately sealed, packed in crushed ice, and analyzed within 30 minutes. Lead II EEG, ECG, and blood pressure were recorded before, during, and after the measurement of CBF. We compared spectral power between the control and FC-23 conditions (p>0.5).

We studied the effect of 60% FC-23 on cerebral venous and arterial oxygen saturation and hemoglobin concentration with a CO-oximeter (model IL482, Instrumentation Laboratory, Lexington, Mass.) at three times during each CBF measurement, and we calculated the oxyhemoglobin content and CMRO2. We evaluated changes in oxyhemoglobin content and CMRO2 using paired t tests.

In nine additional cats, we studied the central nervous system (CNS) and central circulatory effects of 70% FC-23. These cats were prepared similarly to those in which CBF was measured, but the sagittal sinus was not cannulated. Anesthesia and paralysis were achieved with 2 mg/kg ketamine and 0.06 mg/kg pancuronium bromide administered every 30 minutes. Lead II EEG, electrocardiogram (ECG), and cardiac sensitivity to epinephrine were studied during the control condition (inhalation of 30% O2+70% N2) and during inhalation of 70% FC-23; in five of these 9 cats these measurements were repeated during the inhalation of 40% CFC-22. Each study followed a ketamine injection by 15 minutes, and the administration of FC-23 or CFC-22 began 10 minutes before the study. EEG, ECG, and blood pressure were recorded before, during, and after the infusion of 0.1 μg/kg epinephrine in two cats (both received CFC-22) or 1 μg/kg epinephrine in seven cats (three received CFC-22) over 10 seconds. We visualized the EEG tracings from all nine cats revealed increased activity in the theta bin after FC-23 inhalation (Figure 1).

Results

Kety-Schmidt N2O clearance measurements of CBF in 17 cats are presented in Table 1. Control CBF did not differ significantly from that during inhalation of 60% FC-23 either before (p>0.25) or after (p>0.43) adjusting for Paco2. No significant differences were detected in CMRO2 or arterial oxyhemoglobin content between the control and FC-23 conditions (p>0.5).

Visual review of the EEG tracings from all nine cats revealed increased activity in the theta bin after 10 minutes of 70% FC-23 inhalation (Figure 1). Theta and alpha activity in both the frontal and occipital leads increased significantly (p<0.05) during FC-23 inhalation (Figure 2). Spectral power decreased nonsignificantly in the frontal and lateral leads after FC-23 inhalation produced suppression-burst activity indicative of deep anesthesia. In the other three cats, signal amplitude above normal levels was decreased nonsignificantly in the frontal and lateral subdural needle electrodes. All records were visually reviewed, and in the last five cats we analyzed the changes in response to epinephrine using a paired t test. Referential EEG was monitored in the right hemisphere via frontal, occipital, and lateral subdural needle electrodes. All records were visually reviewed, and in the last five cats we analyzed 20–30 4-second epochs of EEG using a fast-Fourier transform algorithm and averaged the spectral power into five frequency “bins” (0–4 [delta], 4–8 [theta], 8–13 [alpha], 13–30 [beta], and 30–60 [gamma] Hz). To each bin we added 1.0 and transformed the result using the natural logarithm. We compared spectral power between the control and FC-23 conditions using a paired t test.

<table>
<thead>
<tr>
<th>Condition</th>
<th>CBF (ml/100 g/min)</th>
<th>Paco2 (mm Hg)</th>
<th>CMRO2 (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38±10</td>
<td>31.6±2.8</td>
<td>3.3±0.9</td>
</tr>
<tr>
<td>FC-23</td>
<td>41±13</td>
<td>32.3±3.3</td>
<td>3.4±0.8</td>
</tr>
</tbody>
</table>

FC-23, trifluoromethane; CBF, cerebral blood flow; CMRO2, cerebral metabolic rate for oxygen. Data are mean±SD.
approximately 4 Hz was greatly decreased (not shown).

Heart rate before epinephrine, arterial blood pressure, and the pressor response to 1 \( \mu \)g/kg epinephrine during 70% FC-23 inhalation did not differ significantly from that during the control condition (Table 2). Inhalation of 40% CFC-22 significantly decreased both blood pressure \((p<0.05)\) and the pressor response to epinephrine \((p<0.05)\). Infusion of 0.1 \( \mu \)g/kg epinephrine in two cats produced little or no effect on blood pressure or heart rate during the control condition or inhalation of 70% FC-23 or 40% CFC-22. No arrhythmias were induced by this dose of epinephrine during either the control condition or inhalation of 70% FC-23. However, during 40% CFC-22 inhalation in one cat 0.1 \( \mu \)g/kg epinephrine elicited a severe arrhythmic reaction (Table 3).

During the control condition, none of seven cats experienced any abnormal alterations in ECG after 1.0 \( \mu \)g/kg epinephrine infusions. The characteristic response to this dose of epinephrine consisted of an initial rise in heart rate, followed by a rise in blood pressure and subsequent bradycardia, with blood pressure gradually returning to normal. During 70% FC-23 inhalation, 1.0 \( \mu \)g/kg epinephrine elicited variable responses (Table 3). Three cats responded as during the control condition, one demonstrated a mild response, and three suffered severe arrhythmic responses (Figure 3). In general, during inhalation of 70% FC-23 arrhythmia in response to epinephrine was transient, lasting only several seconds, in contrast to that during 40% CFC-22 inhalation, when arrhythmia lasted up to 10 times as long.

Two cats suffered prolonged periods of arrhythmia after the withdrawal of CFC-22 (Figure 4). Approximately 1 minute after discontinuing the CFC-22, both animals began a period of spontaneous multifocal ventricular tachycardia with ventricular group beats lasting 2–3 minutes. The onset of arrhythmia coincided with the rise in blood pressure to near control levels.

**Discussion**

In cats\(^1\) CFC-22 increases CBF by 50%, eliminating this agent as a possible NMR indicator. In

---

**FIGURE 1.** Trends in electroencephalogram recorded from referential occipital (left) and frontal (right) leads during control condition (above) and inhalation of 70% trifluoromethane (FC-23) (below) in cats. Increased theta \((4–8\) Hz) and alpha \((8–13\) Hz) activity are apparent in either lead during FC-23 inhalation.

**FIGURE 2.** Bar graph of mean ± SD percentage change in power between control condition and inhalation of 70% trifluoromethane (FC-23) in frontal (shaded bars) and occipital (cross-hatched bars) referential leads in five cats. Only theta \((4–8\) Hz) and alpha \((8–13\) Hz) activity changes significantly \((p<0.05)\) during FC-23 inhalation in either lead. Mean activity in lateral lead changed similarly but nonsignificantly (data not shown).

**TABLE 2.** Effect of FC-23 and CFC-22 on Heart Rate and Blood Pressure, and Responses to 1 \( \mu \)g/kg Epinephrine in Cats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>70% FC-23</th>
<th>40% CFC-22</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{min}^{-1} )</td>
<td>182±24</td>
<td>171±21</td>
<td>170±32</td>
</tr>
<tr>
<td>Change</td>
<td>7 60±30</td>
<td>7 58±23</td>
<td>Obscured by arrhythmia</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{mm Hg} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>9 139±22</td>
<td>9 134±21</td>
<td>5 95±25^*</td>
</tr>
<tr>
<td>Change</td>
<td>7 62±27</td>
<td>7 61±22</td>
<td>3 42±6.3^*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>9 111±20</td>
<td>9 105±21</td>
<td>5 74±26^*</td>
</tr>
<tr>
<td>Change</td>
<td>7 36±20</td>
<td>7 35±18</td>
<td>3 34±10</td>
</tr>
</tbody>
</table>

FC-23, trifluoromethane; CFC-22, chlorodifluoromethane.

^*p<0.05 different from control by paired t test.
by guest on December 29, 2017 http://stroke.ahajournals.org/ Downloaded from

Table 3. Responses Elicited by Epinephrine Infusions During Inhalation of FC-23 or CFC-22 by Cats

<table>
<thead>
<tr>
<th>Cat</th>
<th>Fluorocarbon</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ( \mu g/kg ) epinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>FC-23</td>
<td>Normal sinus rhythm</td>
</tr>
<tr>
<td>2</td>
<td>FC-23</td>
<td>Normal sinus rhythm</td>
</tr>
<tr>
<td>3</td>
<td>CFC-22</td>
<td>Normal sinus rhythm</td>
</tr>
<tr>
<td>4</td>
<td>FC-23</td>
<td>VPCs, ventricular bigeminy, elevated ST</td>
</tr>
<tr>
<td>5</td>
<td>FC-23</td>
<td>VPCs, elevated ST</td>
</tr>
<tr>
<td>6</td>
<td>FC-23</td>
<td>VPCs, multifocal VT, elevated ST</td>
</tr>
<tr>
<td>7</td>
<td>FC-23</td>
<td>VPCs, fusion beats, JER, elevated ST, sinus bradycardia</td>
</tr>
<tr>
<td>8</td>
<td>FC-23</td>
<td>VPCs, ventricular bigeminy</td>
</tr>
<tr>
<td>9</td>
<td>FC-23</td>
<td>VPCs, elevated ST</td>
</tr>
<tr>
<td>1.0 ( \mu g/kg ) epinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FC-23</td>
<td>VPCs, elevated ST</td>
</tr>
<tr>
<td>4</td>
<td>CFC-22</td>
<td>VPCs, fusion beats, JER, elevated ST, sinus bradycardia</td>
</tr>
<tr>
<td>5</td>
<td>FC-23</td>
<td>VPCs, ventricular bigeminy</td>
</tr>
<tr>
<td>6</td>
<td>FC-23</td>
<td>VPCs, fusion beats, JER, elevated ST, sinus bradycardia</td>
</tr>
<tr>
<td>7</td>
<td>FC-23</td>
<td>VPCs, fusion beats, JER, elevated ST, sinus bradycardia</td>
</tr>
<tr>
<td>8</td>
<td>FC-23</td>
<td>VPCs, ventricular bigeminy</td>
</tr>
<tr>
<td>9</td>
<td>FC-23</td>
<td>VPCs, elevated ST</td>
</tr>
</tbody>
</table>

FC-23, trifluoromethane; CFC-22, chlorodifluoromethane; normal sinus rhythm, cardiac rhythm originating from sinus node with normal PR segment and PP duration; VPC, ventricular premature contraction—wide and distorted QRS complex occurring prematurely due to early origination from within ventricular focus; ventricular bigeminy, ventricular premature contractions alternating with normal beats in regular fashion; VT, ventricular tachycardia—multiple (\( \geq 6 \)) ventricular premature contractions occurring together in rapid fashion; sinus bradycardia, markedly slow sinus rhythm (<60 beats/min); JER, junctional escape rhythm—multiple (\( \geq 6 \)) consecutive beats originating from within A-V junctional tissue; fusion beat, complex cardiac cycle resulting from combination of depolarization waves originating within both sinus and ventricular tissues. For description of cardiac conduction abnormalities see Reference 26.

Our EEG data indicate that inhalation of 70% FC-23 causes a mild CNS stimulatory effect (increased power in the theta and alpha bins) while possibly depressing power at higher frequencies. However, the variance of our data is great due to the few cats studied. To better define the effect of FC-23 on the CNS, a more complete study of the FC-23 dose–response relation for EEG needs to be undertaken. We are currently studying this effect further in primates.

Some inhalational anesthetics induce cardiac arrhythmias, and halocarbons increase cardiac sensitivity to the arrhythmogenic potential of epinephrine. Lesions within the pulmonary or cardiovascular systems also increase the potential for arrhythmias during the inhalation of halocarbons. Since an NMR technique to measure CBF may eventually be used in patients with strokes or other disease states that might elevate endogenous catecholamine levels, the tendency of these halocarbons to increase cardiac sensitivity to epinephrine is of great importance. With these factors in mind, we investigated the sensitizing potential of FC-23 in cats.

Except for elevating the ST segment of the ECG, the response to epinephrine during 70% FC-23 inhalation appears to be an enhancement of typical epinephrine-induced cardiac activity. We observed ST segment elevations only following epinephrine infusions during inhalation of 70% FC-23 or 40% CFC-22. Such ST segment elevations have also been observed during inhalation of other halocarbons and probably indicate myocardial ischemia. Generally, halocarbons are believed to influence coronary blood flow by reducing left ventricular perfusion pressure. While 70% FC-23 did not appear to influence arterial blood pressure or the pressor response to epinephrine, an effect on coronary blood flow could arise through a depression of myocardial contractility, which is masked by a general vasoconstrictor effect in the systemic arterial bed. A similar mechanism has been proposed to explain the depression of coronary blood flow following exposure to methylene chloride.

Aviado suggested that halocarbons simultaneously irritate the upper and lower respiratory tracts, thereby increasing vagal tone and sympathetic activity to the heart and triggering cardiac arrhythmias. Opposing sympathetic and parasympathetic activities might explain the arrhythmia we saw after withdrawing 40% CFC-22 from some cats. Varying concentrations of the halocarbon are still present in the blood for minutes after its withdrawal from the inspired gas, especially with the more soluble gases such as CFC-22, and both parasympathetic and sympathetic activity change rapidly. As the gas begins to clear from the blood, its direct effect on myocardial contractility decreases and blood pressure rises, triggering a reflex increase in vagal tone, which could permit the emergence of ectopic ventricular foci and the spontaneous arrhythmias observed. If halocarbons increase sympathetic activity to the heart through contrast, in 17 cats we found no evidence that 60% FC-23 alters CBF, nor did it affect CMRO2 in 10 cats. To our knowledge, there exists only one other report of the effect of FC-23 on CBF. That report studied regional CBF using microspheres in six cats before and during inhalation of 70% FC-23 and suggested that regional CBF in gray tissues increased while that in white tissues decreased during inhalation of FC-23. Opposing trends could result in no change in mean CBF as measured with the Kety-Schmidt N2O clearance technique or the increase in CBF observed during inhalation of 70% FC-23 could be absent during inhalation of 60% FC-23, although we find neither possibility likely. It is more likely that the effect of a small sample size, combined with order and anesthetic effects had greater influence on those results. Apparently, the potential for FC-23 to cause regional changes in CBF needs to be examined more closely.
respiratory irritation, then any alterations in vagal tone could potentiate arrhythmias. Thus, the acute rise in blood pressure following epinephrine infusion, which causes a reflex increase in vagal tone, leads to the increased incidence of arrhythmia we observed.

Pretreatment of animals with β-blockers decreases cardiac sensitivity to halocarbons, although arrhythmias still occur at higher concentrations of the more toxic halocarbons. Halothane directly stimulates adrenergic β receptors in bronchial smooth muscle, and it is likely that trichlorofluoromethane does so too. Trichlorofluoromethane induces peripheral vasodilation, while CFC-22 may affect smooth muscle tone, as evidenced by its effect on CBF. These findings suggest that some halocarbons directly stimulate β receptors.

The absence of cardiac arrhythmogenic tendencies in healthy animals does not necessarily clear a halocarbon for use as an NMR indicator. Rabbits exposed to 30% or 40% CFC-22 for extended periods demonstrate subpleural hemorrhage, excessive mucus, and evidence of pulmonary emphysema and congestion. However, at low inspiration levels for short periods no irreversible effects were seen. Similar observations have been made in our laboratory following extended periods of 40% CFC-22 inhalation. However, animals inhaling 60% FC-23 for as long as 90 minutes failed to exhibit pulmonary damage.

Rats respond to chlorofluorocarbons with a decrease in heart rate, while dogs and primates respond to the same agents with increases in heart rate. This effect is probably attributable to the occurrence of α receptors in the rat heart and represents a species-specific effect. On the other hand, cardiac arrhythmias can be elicited in dogs breathing 5% CFC-22 but not in monkeys or mice breathing 20% CFC-22. This type of variability probably represents a difference in species sensitivity to the gases, a probable contributing factor to which is the difference in

![Figure 3](image-url)  
**Figure 3.** Electrocardiogram (above) and arterial blood pressure (below) from two representative cats during control condition (left) and during inhalation of 70% trifluoromethane (70% FC-23) before (center) and after (right) infusion of 1 μg/kg epinephrine. Following epinephrine infusion in one cat (top) 20-second period of arrhythmia characterized by ventricular premature contractions and elevated ST segment occurred. Similar infusions of epinephrine before and after FC-23 inhalation in this cat were uneventful. In the other cat (bottom) 30-second arrhythmia consisted of ventricular premature contractions alternating with sinus beats and elevated ST segment.

![Figure 4](image-url)  
**Figure 4.** Electrocardiogram (above) and arterial blood pressure (below) in cat shown in top of Figure 3 immediately after discontinuing 40% chlorodifluoromethane (CFC-22) inhalation. Ventricular group beats appeared 1 minute after switch to 30% O₂ + 70% N₂ and continued for 2 minutes. Even after 6 minutes, isolated ventricular premature contractions were still observed.
specie sensitivity to epinephrine. Since FC-23 is a demonstrated (but relatively mild) cardiac sensitizer, further study of its sensitizing potential (preferably in primates) are necessary before administering it to humans who may already suffer from vascular or pulmonary disease.

Acknowledgments

We wish to gratefully acknowledge Dr. Robert Simkins for his assistance in interpreting our electroencephalography records and Mr. Shazad Butt for his extensive technical assistance.

References

30. Govier WC: Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. J Pharmacol Exp Ther 1968;159:82-90

KEY WORDS • cerebral blood flow • nuclear magnetic resonance • cats
Acute toxicity of a nuclear magnetic resonance cerebral blood flow indicator in cats.
C A Branch, J R Ewing, S C Fagan, D A Goldberg and K M Welch

Stroke. 1990;21:1172-1177
doi: 10.1161/01.STR.21.8.1172
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/21/8/1172