

Lack of Whole-body Pharmacokinetic Differences of Halothane Enantiomers in the Rat

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Background: Halothane is made and used as a racemate (an equimolar mixture of *R*- and *S*- enantiomers). This study was initiated to determine whether there were demonstrable enantiomeric differences in the whole-body pharmacokinetics of halothane that might have significance for studies in which racemate is used.

Methods: Adult male Wistar rats were exposed to halothane vaporized in the atmosphere of a closed constant volume chamber supplied with O₂ commensurate with CO₂ production. Concentrations of halothane enantiomers were measured by a specific gas chromatography–mass spectrometry method. Experiments were performed at four initial concentrations of halothane (0.1%, 0.5%, 1.0%, and 1.5% vol/vol). Enantiomeric differences in whole-body pharmacokinetics were assessed indirectly from the relative chamber atmosphere concentrations of halothane enantiomers.

Results: Concentrations of halothane decreased biphasically. The initial more rapid decrease was interpreted as incorporating absorption, distribution, and clearance; the slower decrease was interpreted as principally incorporating metabolic clearance. The ratio of concentrations of the two halothane enantiomers and of the ratios of the respective areas under the con-

centration–time curves remained constant without differing from unity at any time at any concentration of halothane. The dose-normalized areas under the concentration–time curves for the concentrations 0.1%, 0.5%, and 1.0% did not differ; that for 1.5% was significantly greater, suggesting nonlinear clearance, but the values did not differ significantly between enantiomers at any concentration.

Conclusions: As there were no significant differences in concentrations of the two enantiomers in the chamber atmosphere, enantioselectivity was not demonstrated in the whole-body pharmacokinetics of halothane. (Key words: Exposure chamber; gas chromatography; mass spectrometry; stereoisomerism.)

OVER the past 40 yr of use, numerous aspects of halothane pharmacology, pharmacokinetics, and metabolism have been documented in innumerable reports. Halothane (\pm 2-bromo-2-chloro-1,1,1-trifluoroethane), like all other contemporary volatile anesthetic agents with the exception of sevoflurane, is synthesized and administered as a racemate (the equimolar ratio of the *R*(+)- and *S*(-)-halothane enantiomers).

With few exceptions,^{1–6} however, the enantiomeric duality of halothane and the finding that two chemical entities were being administered concurrently, has been ignored. Because enantiomers may have qualitatively or quantitatively different actions, different pharmacokinetics, or different metabolic products, the consequences of treating a racemate as a single entity range from being negligible to the creation of frankly misleading data, depending on the nature and the magnitude of the differences between the enantiomers.

During the 1960s, liver damage after administration of halothane was reported, and subsequent studies concluded that liver damage is linked to halothane metabolism.^{7–9} In humans, as in rats, between \approx 20% and 50% of the halothane that enters the body is metabolized, most of which is metabolized *via* oxidative pathways of the hepatic cytochrome P450 system, with lesser amounts being metabolized *via* reductive pathways and *via* conjugation with glutathione.^{7–9} Although most halothane is metabolized in the liver, evidence has been found for

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extrahepatic metabolism the upper alimentary and respiratory pathways in the rat, corresponding to the presence of cytochrome P450 at these sites.¹⁰

Recent studies have identified the particular cytochrome P450 isoforms involved in metabolism of halothane by liver microsomes *in vitro*.¹¹⁻¹⁶ These studies, using probes for selective inhibition of metabolism of halothane, found that multiple P450 isoforms were involved in oxidative (cytochrome P450 2E1 and 2A6) and reductive (cytochrome P450 2A6 and 3A4) pathways. Neither these nor other recent pharmacokinetic studies of halothane¹⁷⁻¹⁹ have made enantiomeric distinction. Nevertheless, one recent study suggested that metabolic activation predisposing toxicity of halothane may be enantioselective.⁴

The current study was initiated as a first attempt to determine whether there is a demonstrable whole-body pharmacokinetic difference between halothane enantiomers in the rat that may have implications for the interpretation of previous studies and may suggest an additional variable to be incorporated into studies of halothane hepatotoxicity.

Materials and Methods

This study was approved by the Animal Care and Ethics Committee of the host institution. The study involved exposure of rats to (racemic) halothane in a constant volume chamber in which oxygen was supplied commensurate with production of carbon dioxide. Analysis of the chamber atmosphere by gas chromatography-mass spectrometry with a chiral column enabled an index of the rate of loss of the two enantiomers to be determined concurrently after administration of the racemate. To amplify any concentration-dependent effects, the studies were performed at 0.1%, 0.5%, 1.0%, and 1.5% (vol/vol) initial concentrations of halothane in the chamber. Groups of four adult male Wistar rats (body weight, 300–400 g) were used for each exposure, one group with 0.5% and 1.0% and the other with 0.1% and 1.5% concentrations of halothane. Four animals were used at a time to increase the absolute rate of metabolism and thus increase the sensitivity of the study.

Exposure Chamber

The exposure chamber (volume = 19 l) was set up as shown in figure 1; a Poet II gas analyzer (Criticare, Milwaukee, WI) was used to monitor the concentrations of halothane, oxygen, and carbon dioxide in the cham-

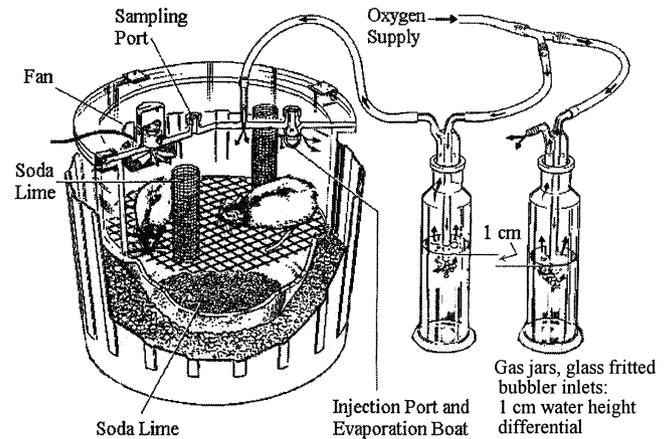


Fig. 1. Experimental setup for determining the chamber atmosphere kinetics of halothane. (Adapted from Reid MA, PhD Thesis, Flinders University, Adelaide, Australia, 1990.)

ber continuously (fig. 1). Before each experiment, approximately 1 kg soda lime (Proisorb; Promedica, Sydney, Australia) was placed in the bottom of the chamber and in the canisters. Four rats were placed into the chamber (when rats were used), the chamber lid was smeared with glycerol to ensure an airtight seal, and then it was sealed in place with bulldog clips. The fan was switched on, the oxygen flow was set so that slow flow of bubbles was seen in both fritted glass diffusers, the Poet II flow rate was set at 50 ml/min, and the chamber atmosphere was recirculated to the chamber to maintain a closed system.

Dose and Sampling Regimens

Volumes of halothane, calculated to give respective initial concentrations of 0.1%, 0.5%, 1.0%, and 1.5% (racemate, vol/vol), were injected through the injection port into the evaporation boat; the system was allowed to equilibrate with the fan running for 15 min while the animals settled. Serial 5- μ l chamber atmosphere samples were then removed using a 25- μ l gas-tight syringe fitted with a repeating adapter (Alltech, Sydney, Australia) at 10-min intervals for up to 5 h. The samples were injected manually into the gas chromatography-mass spectrometer, which had a method run time of 10 min. Each animal exposure experiment was replicated six times at each of the four concentrations of halothane. A series of six replicates also was made at a chamber concentration of 1.0% halothane with no rats in the chamber to determine any losses of halothane resulting from sorption by the soda lime and from sampling by the Poet II monitor.

Halothane Gas Chromatography–Mass Spectrometry Analysis

The analyses were made on a Hewlett Packard 5,890 SERIES II Plus Gas Chromatograph (Palo Alto, CA) equipped with a model 7,673 autosampler and a 5,972 SERIES Mass Selective Detector in EI mode (70 eV). The column was a 40-m trifluoroacetyl substituted β -cyclodextrin capillary column (ChiralDEX-BTA; Alltech, Sydney, Australia) with an ID of 0.25 mm and a film thickness of 0.25 μ m. High-purity helium was used as the carrier gas at 0.5 ml/min. The injector temperature was 200°C, the detector temperature was 250°C, and the oven temperature was isothermal at 20°C using cryogenic control. The Mass Selective Detector was operated in splitless, selective ion monitoring mode using ions at m/z 117 and m/z 198. The dwell time for each ion was 100 ms.

Peak areas of the two halothane enantiomers were measured automatically by the gas chromatography–mass spectrometry software (peak area units = μ V.s) and were used directly for pharmacokinetic analysis. In the absence of authentic samples of the pure *R*- and *S*-halothane enantiomers, it was not possible to assign absolute configurations to halothane enantiomers 1 (retention time, 8.36 min) and 2 (retention time, 8.72 min); hence, the enantiomers were designated by their order of elution. At an initial chamber atmosphere concentration of halothane of 1.0%, the enantiomeric ratio (peak 1:2) was found to be 0.993, with a coefficient of variation of 2.3% and an assay coefficient of variation for both peaks of 2.8% ($n = 8$). The theoretical plate counts for enantiomer 1 and enantiomer 2 were 18,382 and 17,926, respectively, at 0.1% and 19,802 and 18,808, respectively, at 1.5% concentrations of halothane in the chamber. The chromatographic conditions described achieved 99% baseline separation at 0.1% and 97.0% at the 1.5% concentration of halothane in the chamber, with enantiomeric resolutions of 1.43 at 0.1% and 1.50 at 1.5% concentrations in the chamber.

Data Analysis

Methods were chosen to make clear any differences between enantiomers rather than to describe the pharmacokinetics of halothane because the latter incorporates factors for the experimental model/apparatus used (e.g., volume of chamber and soda lime, number and size of animals used, time for chamber halothane equilibration, temperature, gas sampling rate by the Poet II monitor).

Initial concentrations of enantiomers were assigned

values of 50% of the relevant respective nominal concentrations of racemate (which were verified independently by the Poet II monitor). Concentrations of halothane enantiomers in the chamber atmosphere were normalized for the relevant initial concentrations (assigned values of 100% at time 0). Concentration (c)–time (t) curves for halothane in the chamber atmosphere for the experiments without rats were fitted by linear and log-linear regression equations using Statistix 4.1 software (Analytical Software, Tallahassee, FL) to determine the rate of loss of halothane to the system (soda lime sorption plus miscellaneous losses). The preferred solution was judged on the basis of R^2 for each type of equation. The curves for studies with rats were fitted by the biexponential decay equation $c(t) = ae^{-bt} + ce^{-dt}$ using Sigma Plot 2.0 software (Jandel Scientific, San Rafael, CA). The terms a and c are scaling factors and were constrained so that $a + c = 100\%$ at $t = 0$; the terms b and d are exponential hybrid disposition rate constants for which the faster exponent b was assumed to reflect a predominance of distribution and the slower exponent d was assumed to reflect a predominance of metabolic clearance. This was the simplest model consistent with the data and the methods of data collection. This treatment of the data is based on the premise that, in a closed system, any continuing loss of halothane is a consequence of metabolism and thus likens the pharmacokinetic behavior of halothane in the rats to a two-compartment open model, as has been described previously.²⁰ More complex models were not justifiable from either the data or the sampling methods.

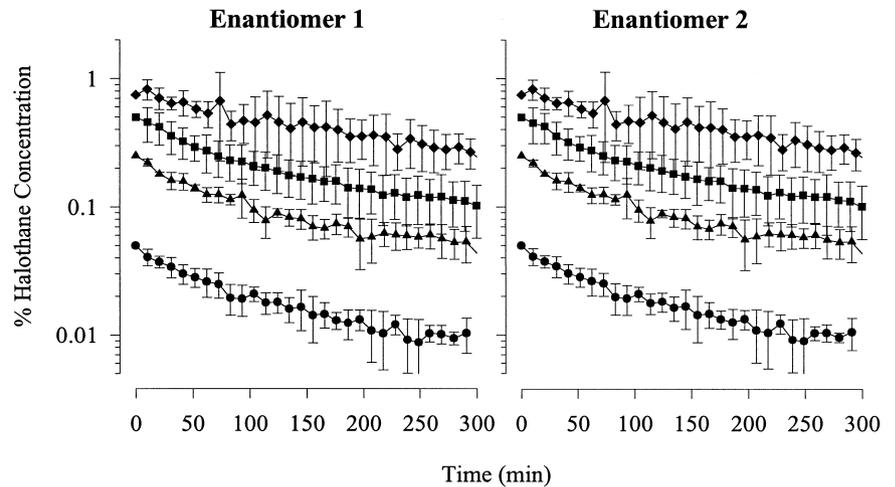
Statistical treatments of data were designed to determine whether there were differences between enantiomers at each concentration of halothane. Enantioselective whole-body disposition of halothane was deemed to occur if the enantiomer ratio of the concentrations in the chamber atmosphere, the ratio of the areas under the concentration–time curves (AUCs; trapezoidal rule) or the ratio of the magnitude of the slower exponents d differed significantly from unity (Student one-sample t test) or there was a difference in relevant mean values (Student t test for paired data). Results comparing different initial concentrations of halothane were analyzed by one-way analysis of variance. Analyses were performed using Statistix 4.1 software.

Results

After being placed in the chamber, the animals moved around for 10–15 min before settling. Hence, 15 min

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Fig. 2. Mean (SD) of the concentrations of halothane in the chamber atmosphere for initial chamber concentrations of 0.1%, 0.5%, 1.0%, and 1.5% (racemate, vol/vol). Initial concentrations of enantiomer were assigned to be 50% of the racemate concentrations. Time 0 was the start of the first sample from the chamber atmosphere, *i.e.*, 15 min after administration of halothane to the rats.



was used as an empirical time to allow for mixing and pseudoequilibrium of the system. At 0.1% and 0.5% concentrations of halothane, the animals slept intermittently for various periods during exposure. At concentrations of 1.0%, they were sedated but easily rousable; at 1.5%, they were anesthetized and took several minutes at the conclusion of the study period to awaken.

There was no loss of halothane from the closed system. Analysis of concentration-time data for halothane in the experiments without rats found that the linear regression equation was $c(t) = 100$ (SD 4.8) $- 0.174$ (SD 0.069) $\times t$ (average $R^2 = 0.66$); that for log-linear regression was $c(t) = 2.01$ (SD 0.02) $- 0.00103$ (SD 0.00057) $\times t$ (average $R^2 = 0.66$). These equations described the loss of halothane equally well for both enantiomers as judged by the finding that the R^2 values were not significantly different ($P = 0.96$). Thus, the losses attributable to sampling by the Poet II monitor circuit and by sorption by soda lime at the initial chamber concentration of 1.0% halothane averaged 0.17% (SD 0.07) per minute or 0.0010% (SD 0.0006) of the chamber concentration per minute, respectively, as revealed by linear and log-linear regression.

Figure 2 shows the chamber atmosphere concentration-time graphs of both enantiomers at each of the initial concentrations of halothane. After the rats were introduced to the system the concentrations in the chamber atmosphere decreased in a biphasic manner and were represented adequately by the biexponential decay equations for all except two cases at 0.1% and at 1.5%, at which the decrease was essentially monophasic. Using one-sample t tests and one-way analysis of variance, the mean ratio of concentrations of enantiomer did not differ to unity at any concentration of halothane. A

representative example at 1.0% (vol/vol) initial chamber concentration of halothane for enantiomers 1 and 2 is shown in figure 3. This gave biexponential curve fits with equations $c(t) = 68.3e^{-0.023t} + 31.7e^{-0.0016t}$ for enantiomer 1 and $c(t) = 66.7e^{-0.023t} + 33.3e^{-0.0019t}$ for enantiomer 2. Values for the slower exponent d are given in table 1. Neither the enantiomer ratios of the AUCs nor the slower exponents d differed to unity for any concentration of halothane.

Figure 2 suggests that the concentrations of halothane for 1.5% differ disproportionately to the three lower concentrations, raising the possibility of saturation kinetics of halothane occurring between 1.0% and 1.5%. This is borne out by the finding using one-way analysis of variance of a disproportionately greater dose-normalized AUC at 1.5% (table 1) than at the lower concentrations. The value of the disposition rate constant d tended to increase with increasing initial concentration of halothane (table 1), but the groups were not significantly different (one-way analysis of variance, $P = 0.15$).

As the same animal groups were treated to multiple exposures of halothane, an order of exposure effect could have suggested enzyme induction. The AUC data were scrutinized for an effect of order, but none was found for any concentration of halothane.

Discussion

With current methods, it is not usually possible to predict from first principles to what extent drug enantiomers interact differently with endogenous chiral molecules; the differences in their kinetics and dynamics must be determined experimentally. By analyzing for

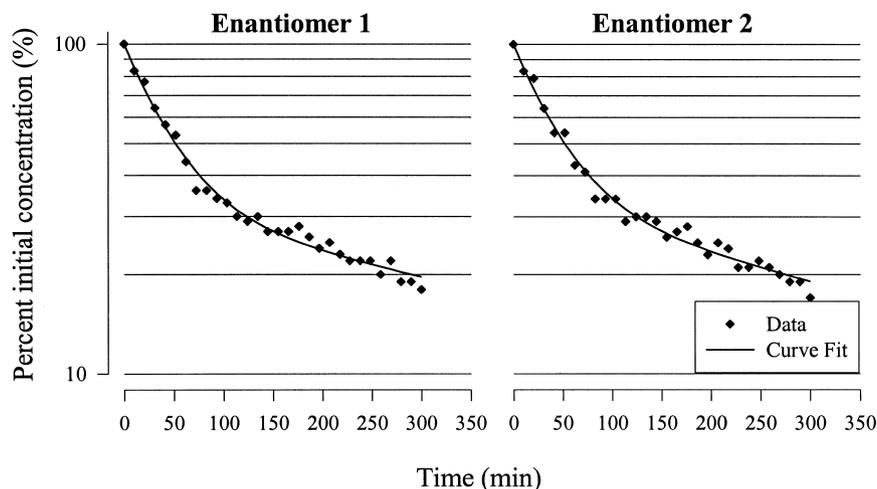


Fig. 3. Representative results for chamber atmosphere concentration-time graphs of (left) enantiomer 1 and (right) enantiomer 2, showing the fit of the biexponential decay equation to the data.

concentrations in the chamber atmosphere of *R*- and *S*-halothane over time, any differences between enantiomers in the rates of whole-body uptake and consumption by the rats could be determined. As the ratio of the concentrations of halothane enantiomer remained constant at unity over time for all concentrations of halothane in the chamber atmosphere, it was concluded that no significant differences in the whole-body pharmacokinetics of the halothane enantiomers were detectable in the rats.

Further analysis using the rates of change of concentrations of enantiomer determined from the curve fit parameters gave indices of the component rates of distribution and metabolism. Because the pharmacokinetics were being assessed indirectly, *i.e.*, by sampling outside of the body, it was necessary to assume that vaporization and mixing of halothane in the chamber is not enantioselective and that absorption of halothane occurs at the same first order rate for both enantiomers. The former assumption is verified by the lack of difference in concentrations of halothane in the chamber atmosphere in the experiments without rats. The latter is logical in that

it would be expected that the respective rates of absorption and distribution of the two enantiomers would be similar as the blood/gas and tissue/blood partial pressure gradients equilibrate. The net rate of equilibration, reflected in the faster disposition rate constant *b*, would thus be a complex function involving the physicochemical properties of halothane, the ventilation and perfusion of the rats, and the properties of the exposure chamber and contents. These values also were not found to be different between enantiomers.

Any difference in the rate of change of concentrations of enantiomer in the chamber atmosphere after equilibration would be expected to be attributable principally to a difference in metabolic clearances and reflected in the slower disposition rate constant *d*. This also was not found, providing indirect evidence for there being no detectable enantioselectivity in the rate of halothane metabolism by Wistar rats. Hence, there is a reasonable expectation that previous metabolic and pharmacokinetic studies of halothane performed without enantiomeric differentiation in this species, at least, are not likely to be markedly in error. Beyond any consumption

Table 1. Mean [SD] of the Areas under the Curve for Halothane Enantiomers 1 and 2 and the Slow Disposition Constant *d* at 0.1, 0.5, 1.0 and 1.5% (vol/vol) Halothane Initial Chamber Concentrations (n = 6 at each concentration)

Initial Halothane Concentration (vol/vol %)	AUC			Slow Disposition Constant <i>d</i>		
	Enantiomer 1 (% · min)	Enantiomer 2 (% · min)	Ratio (Enantiomer 1:2)	Enantiomer 1 (min ⁻¹) × 10 ³	Enantiomer 2 (min ⁻¹) × 10 ³	Ratio (Enantiomer 1:2)
0.1	5.5 [1.1]	5.6 [1.0]	0.99 [0.01]	3.2 [2.3]	3.34 [2.3]	0.91 [0.17]
0.5	28.1 [3.3]	27.9 [3.4]	1.01 [0.01]	3.5 [1.9]	3.6 [1.9]	0.96 [0.06]
1.0	61.1 [18.5]	61.2 [18.2]	1.00 [0.02]	5.7 [5.9]	5.7 [5.8]	1.00 [0.03]
1.5	137.7 [42.0]	137.5 [41.2]	1.00 [0.01]	5.8 [4.8]	5.5 [4.7]	1.10 [0.19]

AUC = area under the curve.

by the rats, it is noted that the carbon dioxide absorber soda lime induces a dehydrofluorination of halothane with a loss of chiral center.²¹ This chemical degradation is not stereoselective as shown by the rates of loss from the chamber without rats also being the same for both enantiomers.

The association between halothane metabolism to reactive trifluoroacetyl chloride and liver injury²²⁻²⁴ was explored recently enantiomerically.⁴ In that study, the racemate and separate enantiomers of halothane in sesame oil were injected intraperitoneally into mice and the livers examined semiquantitatively 24 h later for trifluoroacetyl-protein adducts.⁴ The authors found that two to three times more adduct derived from oxidative metabolism of *R*-halothane than from *rac*- or *S*-halothane.⁴ There are several possible explanations for the apparent variance of this result with the current data. There may be a species difference between mice and Wistar rats in the enantioselectivity of their metabolism of halothane. The modes of administration, intraperitoneal injection compared with inhalation, may have influenced the outcome; intraperitoneal injection would present the liver with higher concentrations of halothane arising from direct portal venous delivery so that metabolite production would be largely circumscribed by hepatic enzymic capacity for metabolism. As the authors did not estimate the fraction of the doses that were metabolized in that manner, it is not possible to evaluate its quantitative contribution to the whole-body pharmacokinetics of halothane and whether it would be detectable by a whole-body method if it did occur. Further studies with both preparations involving hepatic enzyme induction and inhibition to probe enantioselectivity of metabolism, as well as mass balance with specific metabolites could yield noteworthy results.

Our study found indirect evidence for a lack of significant whole-body pharmacokinetic differences between the halothane enantiomers. Although the enantiomers could have been metabolized at different rates through different pathways to different products, this would have necessitated that the metabolism of each enantiomer occur at the same net rate. In a complex metabolic process, it is unlikely that two enantiomers are metabolized by different pathways yielding the same net rate. Studies to further investigate this point require the separate halothane enantiomers. The question of enantioselectivity in the hepatotoxicity of halothane from whole-body inhalation exposure thus remains open.

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