Title : HOW REACTIVE ARE THE REDUCTIVE METABOLITES OF HALOTHANE?

Authors : D. D. Denson, Ph.D. and D. J. Ford, Ph.D.

Affiliation: Department of Anesthesia, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Introduction. The question of whether hepatotoxicity is associated with halothane anesthesia remains unresolved. The discovery of a reductive metabolic pathway for halothane has resulted in the theory that reductive metabolites may be responsible for the suspected hepatotoxicity. While three reductive metabolites have been isolated either in the expired breath of animals[1] (and now man)[2] or as conjugates in human urine[3], the reactivity of these compounds has not been studied. This is the first report of the reactivity of two of these metabolites, 2-bromo-2-chloro-1,1-difluoroethylene (BCDFE) and 2-chloro-1,1-difluoroethylene (CDFE).

Methods. CDFE and BCDFE were obtained from PCR, Inc., Gainesville, Florida under a custom synthesis contract. Both compounds were found to be > 99% pure by gas liquid chromatography (glc). Partition coefficients were determined by the method of Larson, et al[4]. Each determination presented in Table 1 is the value after four hours at 37°C. However, with both BCDFE and CDFE equilibrium was established after 10 to 15 minutes depending on the medium. Kinetic experiments were conducted in 12cc vacutainer tubes. The appropriate volume per cent concentration of BCDFE or CDFE was introduced and analyzed by glc. 0.5cc of the medium to be studied was introduced by syringe and the tubes incubated at 37°C. Each experiment was assayed for either fluoride or free sulfhydryl and olefin at 0, 10, 20, 30, 40, 50, and 60 minutes. All reactions were found to be linear over this range. Pseudo-first order rate constants (Table 2) were derived from plots of ln A/A_o vs time ($r \geq 0.997$). The rate of hydrolysis was determined by measuring the production of fluoride using a specific ion electrode. The rate of reaction of sulfhydryl groups was determined by following the rate of disappearance of free SH.

Results. Partition coefficients suggest that CDFE is relatively insoluble while BCDFE is highly soluble in both whole blood and oil.

Table 1: Partition Coefficients (Mean ± SE)

	BCDFE	CDFE
Plasma:Gas	1.96 ± 0.06	2.02 ± 0.14
Blood:Gas	10.6 ± 0.6	4.8 ± 0.4
Oil:Gas	113 ± 5.1	12.0 ± 1.0

We examined both the hydrolysis of BCDFE and CDFE in a number of media and the reactivity of BCDFE and CDFE with L-cysteine and reduced glutathione. In all cases the hydrolysis reactions were first order and the rate is 1% per hr. for BCDFE and 0.01%/hr. for CDFE.

Table 2: Pseudo First Order Rate Constants ($sec.^{-1}$)[1]

Reactants (media + olefin)	BCDFE	CDFE
Plasma + 2V%	$2.35 \pm 0.2 \times 10^{-6}$	$5.6 \pm 0.8 \times 10^{-8}$
Plasma + 10V%	$3.02 \pm 0.2 \times 10^{-6}$	$6.1 \pm 0.4 \times 10^{-8}$
L-Cysteine + 10V%	$1.7 \pm 0.4 \times 10^{-4}$	-0-
(SH) Glutathione + 2V%	$2.02 \pm 0.22 \times 10^{-4}$	-0-
(SH) Glutathione + 10V%	$1.92 \pm 0.20 \times 10^{-4}$	-0-
(SH) Glutathione + 20V%	$1.77 \pm 0.20 \times 10^{-4}$	-0-
(SH) Glutathione + 40V%	-----	-0-

[1] mean ± SE for $n \geq 6$ runs

Conditions for the glutathione reactions were established in such a way as to reflect pseudo-first order kinetics enabling us to compare the rate of hydrolysis with the rate of reactions with a sulfhydryl group.

Discussion. Our data suggest that the rate of hydrolysis is independent of solubility since no change in rate was found in water, plasma, whole blood, or microsomal protein. BCDFE reacts with sulfhydryl groups 50 times faster than it is hydrolyzed suggesting that conjugation rather than hydrolysis is an important route for clearing BCDFE from the body. From the partition coefficient and kinetic data we conclude the following: 1) The low solubility of CDFE should allow rapid clearance via the lungs and hence, this compound should be readily detectable. BCDFE which is much more soluble in both blood and oil would be difficult to detect and would tend to remain in the body for somewhat longer periods of time. 2) The rapid rate of reaction of BCDFE with glutathione and the lack of reactivity of CDFE with glutathione help explain the isolation of the BCDFE cysteine conjugate but not the CDFE cysteine conjugate from human urine.[3] This study is an important first step in understanding the clinical implications of exposure to BCDFE and CDFE.

References.

1. Mukai S, Morio M, Fujii K, Hanaki C: Volatile metabolites of halothane in the rabbit. Anesthesiology 47:248-251, 1977
2. Sharp JH, Trudell JR, Cohen EN: Volatile metabolites and decomposition products of halothane in man. Anesthesiology 50:2-8, 1979
3. Cohen EN, Trudell JR, Edmunds HN, Watson E: Urinary metabolites of halothane in man. Anesthesiology 43:392-401, 1975
4. Larson CP, Eger EI, Severinghaus JW: The solubility of halothane in blood and tissue homogenates. Anesthesiology 23:349-355, 1962