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Title : Toxicity of Ultraviolet (UV) Irradiated Halothane in Mice

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Introduction. Halothane toxicity has been reported to occur both following anesthesia and after chronic exposure to trace levels. Its metabolism to reactive intermediates via the hepatic microsomal Mixed-Function Oxidase (MFO) system is required for toxicity. The increased toxicity of uv-irradiated halothane has also been reported. We have extended the investigation of irradiated halothane to the identification of decomposition products and mechanisms of toxicity.

Methods. Male and female CD1 mice, 7 to 10 weeks old, were exposed for 90 minutes in a 20 l chamber to O_2, to halothane, or to irradiated halothane. At 2 l/min, O_2 was successfully passed through a Fluotec vaporizer and a 2 inch stainless steel tube in which a Westinghouse germicidal lamp (#782L-30) was mounted and then diluted to 1.3% halothane in 6 l O_2/min to enter the chamber. The glutathione (GSH) content of livers, washed free of blood with 5 mM EDTA in 1.15% KCl, was determined immediately after exposure. Serum enzyme measurements, lung wet:dry ratios, and microsome isolations for determinations of cytochromes P_{450} and b_5 and aminopyrine demethylase activity were performed 24 hours after exposure. Gas chromatography (GC) was performed at 25° C in a Varian 3700 fitted with a 6 ft column containing 10% SP 2100 on Chromsorb G-HP (80/100). Air, O_2 or N_2 were used at the diluent gas to study the nature of the decomposition products.

Results. Eight decomposition product peaks appeared in GC profiles of halothane irradiated in N_2 or air. Three were identified as CF_3-CH_2Cl, CF_2=$CHCl$, and CF_3-$COCl$. Additionally, Br_2 and F^- were identified spectrally and via ion specific electrode, respectively. Irradiation in O_2 produced three products. Two unidentified peaks and CF_3-$COCl$ representing 0.08 and 0.22% of the total area, respectively. The content of CF_3-$COCl$ decreased with decreasing O_2 in the irradiation atmosphere. Exposure of male mice (Table I) to 1.3% irradiated halothane led to 2.3- and 3.8-fold increases, respectively in SGOT and SGPT. Similarly, female mice showed 1.5-fold increases in both enzymes. Halothane and halothane + uv exposure, respectively, decreased Cyt P_{450} and b_5 contents 5 and 15-20% in male mice (Table II). Exposure of female mice resulted in no significant changes from the control levels of 0.711 ± 0.058 and 0.372 ± 0.049 nMole Cyt P_{450} and b_5/mg protein, respectively. Aminopyrine demethylase activity was 9.8 ± 0.2, 9.9 ± 1.3, and 7.3 ± 0.6** nMole formaldehyde/min/mg protein, respectively for male mice exposed to O_2, halothane, and halothane + uv. Total hepatic GSH was lowered 28% in male mice exposed to uv irradiated halothane (Table III). Breathing difficulties were observed in both sexes after exposure to halothane + uv. Pulmonary edema was indicated only in male mice by comparison of lung wet:dry ratios. Values of 4.4 ± 0.1, 4.6 ± 0.5, and 5.2 ± 0.2** for males and 4.3 ± 0.1, 4.7 ± 0.3, and 4.2 ± 0.1 for females resulted from exposure to O_2, halothane, and halothane + uv, respectively.

TABLE I. EFFECT OF IRRADIATED HALOTHANE ON SERUM TRANSAMINASES

EXPOSURE	SGOT†	% CONTROL	SGPT †	% CONTROL
O_2	52.3 ± 3.0 (12)	100	25.0 ± 2.4 (12)	100
HALOTHANE	59.9 ± 6.0 (11)	115	25.5 ± 2.9 (10)	102
HALOTHANE + UV	121.8 ± 12.2 (9)**	233	94.2 ± 21.5 (9)**	377

† MEANS ± STANDARD ERROR; UNITS = SIGMA - FRANKEL UNITS/ML SERUM

TABLE II. EFFECT OF IRRADIATED HALOTHANE ON HEPATIC MICROSOMAL HEMOPROTEINS

EXPOSURE	CYTOCHROME P_{450} †	% CONTROL	CYTOCHROME b_5 †	% CONTROL
O_2	1.016 ± 0.030 (14)	100.0	0.450 ± 0.023 (14)	100.0
HALOTHANE	0.963 ± 0.036 (15)	94.8	0.434 ± 0.016 (15)	96.4
HALOTHANE + UV	0.819 ± 0.044 (15)**	80.6	0.390 ± 0.022 (15)*	86.7

† MEANS ± STANDARD ERROR; UNITS = NMOLE / MG PROTEIN

TABLE III. EFFECT OF IRRADIATED HALOTHANE ON HEPATIC GLUTATHIONE CONTENT

EXPOSURE	GSH + GSSG †	GSSG †
O_2	48.7 ± 3.3 (5)	9.9 ± 0.6 (5)
HALOTHANE	63.0 ± 9.5 (5)	16.4 ± 2.6 (5)*
HALOTHANE + UV	35.2 ± 4.6 (5)*	6.8 ± 1.6 (4)

(N) = NUMBER OF ANIMALS
† MEANS ± STANDARD ERROR ; UNITS = UG / G WET WT. LIVER
* P < 0.05; ** P < 0.01

Discussion. The uv-irradiation of halothane results in its decomposition to products with pulmonary and hepato-toxicity in mice. Decreased aminopyrine demethylase activity parallels the destruction of the MFO components, Cyt P_{450} and b_5, suggesting a requirement for bioactivation of uv-irradiated decomposition products for toxicity. By comparison, female mice were less susceptible to toxicity from these products than males. Since the MFO system of females is generally less active than males, decreased bioactivation is the likely cause for decreased toxicity. Hepatic GSH depletion suggests its role in the metabolism of halothane decomposition products. Relative toxicities of halothane irradiated in O_2, N_2, and air are under current investigation.

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