TITLE: HALOTHANE INHIBITS OXIDATIVE DRUG METABOLISM IN INTACT RATS

AUTHORS: L. E. Bell, M.D., and J. T. Slattery, Ph.D.

AFFILIATION: Department of Anesthesiology, and Department of Pharmaceutics, University of Washington
Seattle, Washington 98195

Introduction. Inhalation anesthetics have been shown to substantially diminish the clearance of drugs eliminated by hepatic routes. In vitro studies suggest that inhibition of oxidative metabolism may contribute to this effect, but the relative contribution of enzyme inhibition and decreased hepatic blood flow in an intact animal is not known. Understanding the mechanism of the interaction is important in developing the ability to predict what class of drugs (based on pharmacokinetic characteristics) will be subject to the effect. The purpose of the present study was to determine the influence of halothane administration on the in vivo hepatic intrinsic clearance (Cl_{int}) of diazepam.

Methods. Male Sprague Dawley rats were prepared under pentobarbital anesthesia. A portal vein catheter (PE10) was inserted through a splenic vein and the spleen removed. A biliary catheter (PE50) and femoral artery catheter (PE50) were also inserted and the wounds closed. A rectal thermistor was placed and temperature was controlled at 38 ± 1/2°C with heating pad and lamp. Saline was administered at approximately 10 cc/hr to maintain urine flow and blood pressure. The animals were allowed to recover for two hours at which time they were awake and exploring the cage.

A loading dose of 0.25 mg/kg of ^{14}C-diazepam was given via the portal vein catheter followed by a constant infusion at 0.25 mg/kg/hr which was continued for the duration of the study. After two hours of diazepam infusion, 8 rats (group H) were given 1% halothane in oxygen by mask. A control group of 6 rats (group C) breathed air. At two hours (early), four hours and five hours (late) arterial blood samples were taken for analysis. Bile was collected in 15 minute aliquots. The experiment was terminated at 5 hours at which time blood gases and hematocrit were measured. Blood and bile were assayed for diazepam, (D), N-desmethyl diazepam, (ND), 3-OH-diazepam (3OH) and phenyl-OH-diazepam, (POH) by TLC separation and scintillation counting.

Data Treatment. Intrinsic clearance of diazepam was calculated as I/C_{ss}(1) where I = portal infusion rate and C_{ss} is steady state plasma concentration of diazepam. Steady state was confirmed by comparison of early and late serum concentrations of D and metabolites in group C, and 4 and 5 hour concentrations in group H. The ratio of formation clearance to elimination clearance Cl_f/Cl_e for each metabolite was calculated according to:

$$Cl_f/Cl_e = C_{ssm}/C_{ssd} \quad (2)$$

where C_{ssm} and C_{ssd} are steady state plasma concentration for metabolite and diazepam respectively. Formation clearance is the fraction of diazepam clearance contributed by the pathway which forms a specific metabolite.

C_{ss} and Cl_{int} values were compared by Student's *t* test of log transformed data because of skewness. The ratios Cl_f/Cl_e and L/E (late/early) were compared by *t* test of nontransformed data. Comparisons within treatment groups were by paired analysis; those between groups were unpaired. Early and late refer to pre-halothane and halothane periods in group H, and corresponding periods in group C.

Results. A mild respiratory acidosis occurred in group H. BP's and Hct's were not significantly different between groups. Diazepam and metabolite concentrations were not significantly different between 4 and 5 hours, indicating that steady state was reattained following exposure to halothane. Concentrations at these times were averaged (late collection).

The steady state plasma diazepam concentration increased significantly in group H by 75% after exposure to halothane; all metabolites increased significantly in group H as well. There was no change in group C over the same period. Cl_{int} decreased by 42% during halothane administration (Table 1)

Cl_f/Cl_e ratios were unchanged for ND and POH in both groups, but was significantly higher for 3OH in the halothane treated group. Biliary metabolite fractions remained unchanged in both groups.

Discussion. Intrinsic clearance reflects the drug metabolizing activity of the liver and is not influenced by changes in hepatic blood flow or volume of distribution. This study demonstrates in the rat that halothane can significantly inhibit drug metabolism in vivo.

The difference in Cl_f/Cl_e for 3OH indicates that halothane may selectively inhibit its elimination pathways more than its formation. Since 3OH undergoes further oxidative metabolism as well as conjugation prior to elimination we cannot pinpoint which pathways are differentially affected.

References.

1. Pang, K.S. and Rowland, M.: J. Pharmacokin Biopharm 5:625-652 (1977)
2. Lane, E.S. and Levy, R.H.: J. Pharm Sci 69:610-612 (1980)

Table 1. Effect of halothane anesthesia on the pharmacokinetics of diazepam and its metabolites in the rat[1]

	C_{ss}, ng/ml			Cl_f/Cl_e			Cl_{int}, ml/min		
	Early	Late	Ratio, L/E	Early	Late	Ratio, L/E	Early	Late	Ratio, L/E
Control									
D	6.1	5.9	1.03±0.15				433	432	1.04±0.13
ND	2.7	2.5	0.93±0.10	0.61	0.57	0.92±0.05			
POH	4.0	3.8	0.91±0.08	1.1	0.96	0.92±0.10			
3OH	4.1	3.3	0.83±0.08	1.0	0.78	0.82±0.09			
Halothane									
D	7.9	13.2[2]	1.76±0.11[3]				452	241[2]	0.58±0.03[3]
ND	3.1	5.9[2]	2.12±0.25[3]	0.57	0.68	1.16±0.16			
POH	3.8	6.0[2]	1.60±0.15[3]	1.0	0.87	0.86±0.10			
3OH	3.6	8.0[2]	2.12±0.18[3]	0.63	0.76[2]	1.25±0.11[3]			

[1]Mean value, n-6 (control), n=8 (halothane), ± SE for ratios.
[2]$p<0.05$ vs. early. [3]$p<0.05$ vs. control.