SHORT-TERM METABOLIC ETHANOL TOLERANCE IN DOGS

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SUMMARY

Metabolic ethanol tolerance was studied in a cohort of five dogs with ethanol challenge repeated weekly over a 7-week period. During the 7-week period, the area under the blood alcohol versus time curve (AUC) increased slightly while the rate of ethanol elimination also increased slightly. During the repeated ethanol dosing, ethanol absorption shifted from approximately equal absorption in the stomach and intestine to three-fold more absorption in the intestine than in the stomach. The likely cause of the shift in absorption site was probably a concomitant change in gastric emptying that occurred with repeated dosing. This shift is significant since ethanol absorption in the small intestine has been shown to be over six-fold more rapid than ethanol absorption in the stomach.

KEY WORDS

ethanol, tolerance, simulation, gastric emptying

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INTRODUCTION

Vogel-Sprott reported, "One characteristic that correlates with alcohol abuse is a high degree of tolerance for the drug. Thus it is often thought that tolerance may increase the risk of alcohol abuse because alcohol consumption would have to increase to reinstate the effects initially attained by lower doses." /1, p.ix/ Alcohol tolerance can be classified in two ways depending on how the tolerance is manifest. Acute tolerance refers to the observation that the intensity of the effect of a given blood alcohol concentration (BAC) is typically more pronounced when blood alcohol levels are rising than when they are falling /2/. Chronic tolerance occurs when re-administration of the same dose of alcohol produces less of an effect /1, p.14/. Biologically, the basis of alcohol tolerance can be both dispositional (i.e. pharmacokinetic, metabolic), and functional (i.e. pharmacodynamic, psychological, learned). The focus of the research reported here is metabolic tolerance, the reduction in the effect of a drug owing to an increased rate of drug elimination.

Numerous researchers have explored the importance of metabolic ethanol tolerance. Early on, Newman and Lehman /3/ investigated acquired ethanol tolerance in a cohort of five dogs by measuring the dogs' blood alcohol response before and after a 97-day period when the dogs consumed alcohol (10% w/v) twice per day. By the end of the 97-day period the dogs' average rate of ethanol elimination had increased from 0.0029 $mg/cm^3 \cdot min$ to 0.0032 $mg/cm^3 \cdot min$. Newman and Lehman speculated that this 10.3% increase in the dogs' mean ethanol elimination rate was likely not statistically significant. However, working in rats, Lieber and DeCarli /4/ found that feeding ethanol increased the microsomal ethanol oxidizing system (MEOS) activity by 29.2% in males and 122.0% in females. Kater *et al.* /5/ measured the rate of blood ethanol clearance from human alcoholic volunteers using repeated daily dosing over 4 days; day one and day two the dose was administered orally, and days three and four the dose was intravenous (i.v.). With oral dosing, ethanol elimination increased by 3.6 mg/100 ml per hour (SD ± 3.8) and with i.v. dosing ethanol elimination increased by 2.2 mg/100 ml per hour (SD ± 2.7). Kater *et al.* speculated that induction of the MEOS was the likely cause of increased ethanol elimination. Miceli and Magnen /6/ reported that over a period of 16 days with daily injection of ethanol, rats' ethanol

elimination rate increased from 0.436 mg/ml·h to 0.535 mg/ml·h, an increase of 22.7%.

In a study using rats bred to obtain a line whose central nervous system (CNS) was most affected (MA) by alcohol, and a line whose CNS was least affected (LA) by alcohol, Khanna *et al.* /7/ found that after 4 weeks of daily ethanol dosing, both LA and MA female rats' ethanol metabolic rate (EMR) (mg/kg body weight/h) increased by 20%, whereas after 6 weeks daily dosing LA males' EMR increased by 26% and MA males' EMR increased by 89%.

Rachamin *et al.* /8/ maintained rats on an alcohol diet for a period of 4-5 weeks. During the last nine days of the period, 6-n-propyl-2-thiouracil (PTU), which reduces hepatic oxygen consumption, was administered in the rats' regular meal. The rats' rate of ethanol metabolism (mmoles/kg body weight/h) was determined by intraperitoneal ethanol injection (2.5 g/kg) with subsequent blood ethanol determination. Ethanol metabolism in ethanol-fed rats was 49% greater than that of controls; the increase in ethanol metabolism was accompanied by 31% increase in alcohol dehydrogenase (ADH) activity measured *in vitro*. PTU administration for nine days reduced the rate of ethanol metabolism by 31% in ethanol-fed rats and very little in controls. Based on these results, Rachamin *et al.* concluded that re-oxidation of NADH is the limiting step in ethanol metabolism; they further concluded that no MEOS induction was observed in female rats in this study, although MEOS induction was observed in male rats in a previous study /9/.

In a study of ethanol clearance rate (mg/dl/h) in twins, Wilson and Nagoshi /10/ reported no evidence of metabolic tolerance based on a 1-month test-retest protocol. After 4-6 weeks of ethanol intake and 48-50 weeks of chronic ethanol intake, Sancho-Tello *et al.* /11/ demonstrated increases in ethanol elimination rates of 26% and 42%, respectively.

Finally, in an excellent historical review, Kalant /12/ concluded that the literature is ambiguous relative to the significance of metabolic ethanol tolerance. Therefore, it appears that approaches not utilized previously might help provide additional insight into the question of metabolic ethanol tolerance.

The overall objective of the present study was to evaluate metabolic ethanol tolerance in fasted dogs over a period of 7 weeks. Specific aims were: (1) to measure blood ethanol concentrations from

a cohort of five fed dogs challenged weekly with a single bolus oral dose of ethanol; (2) to assess the area under the blood alcohol concentration (BAC) versus time curve (AUC); (3) to assess the rate of blood ethanol disappearance estimated from the straight line descending portion of the BAC versus time curve; (4) to use Monte Carlo simulation with an ethanol pharmacokinetic model and the measured blood ethanol concentrations to obtain weekly values for model parameters; and (5) to use the simulation results to estimate weekly values for AUC and the relative fraction of ethanol absorbed in the stomach and intestine, respectively.

MATERIALS AND METHODS

Ethanol dosing and blood ethanol determination

Each of five mongrel dogs was fasted for 24 hours prior to ethanol dosing. Each dog received 1 g ethanol/kg body weight administered as a gavage of 20% w/v ethanol solution.

Each dog was shaved and swabbed with Betadine solution at the venipuncture site prior to blood sampling. Blood (3 ml) was taken from a catheter placed in the saphenous vein for the duration (6 h) of the experiment. A single blood sample (3 ml) was taken from each dog before the ethanol dose (0 min). Additional blood samples were taken at 10, 20, 30, 40, 60, 80, 100, 120, 180, 240, 300, and 360 minutes after ethanol dosing. Blood was mixed with 7.5 mg solid sodium fluoride and 6.0 mg potassium oxalate to prevent coagulation. Samples and reagents were maintained on ice unless otherwise noted. Standards (1.0 ml) were prepared by mixing 0.9 ml of anticoagulated, ethanol-free blood with ethanol (Aaper Alcohol Co., Shelbyville, KY) solution yielding standard solutions of 0, 2, 4, 6, 12.5, 25, and 60 mM ethanol. Each standard or sample (1 ml) was then mixed with 0.5 ml perchloric acid (1 N PCA, 20 mM thiourea) (PCA) and centrifuged. Supernatants of 0.6 ml were sealed in glass vials for head-space gas chromatography according to Whitmire and Whitmire /13/.

Experimental analysis

Experimental AUC values (mM-min) were obtained by Simpson's rule integration of the BAC versus time curve. The elimination rate

(mM/min) was the slope of the linear region of the descending portion of the BAC versus time curve determined by linear regression.

Ethanol pharmacokinetic model

Earlier we reported an ethanol pharmacokinetic model which accounted for the following processes /14/:

1. The distribution volume for ethanol is the total body water.
2. The distribution volume for ethanol is well mixed; therefore, the blood and the extravascular space excluding the stomach and small intestine are essentially equilibrated.
3. The stomach is a separate compartment.
4. Ethanol elimination from the total body water can be described as a Michaelis-Menten rate expression at the ethanol concentration of interest. Model parameter V_{max} characterizes the metabolic rate.
5. Ethanol transport occurs in either direction by passive diffusion through the stomach wall. Model parameter $ks \cdot Am$ characterizes ethanol absorption in the stomach.
6. Ethanol transport occurs in either direction by passive diffusion through villi of the small intestine. Model parameter $kd \cdot Al$ characterizes ethanol absorption in the intestine.
7. Ethanol can flow from the stomach to the duodenum through the pyloris. Model parameter α characterizes gastric emptying of ethanol into the small intestine.

Stomach

The diffusion model for the stomach consists of normal circulation flowing into a well-mixed vascularized mucosa. Ethanol diffuses across an epithelial layer between the well-mixed mucosa and the stomach lumen. The model parameter characterizing ethanol diffusion in the stomach mucosa is $ks \cdot Am$ which is the product of the mass transfer coefficient for ethanol, ks (cm/min) and the area available for ethanol transport, Am (cm^2).

Small intestine

The diffusion model for the small intestine consists of normal circulation containing ethanol flowing through intestinal villi. Ethanol

diffuses across an epithelial layer between the villi and the small intestine lumen. The model parameter characterizing ethanol diffusion in the small intestine is $kd \cdot Al$ which is the product of the mass transfer coefficient for ethanol, kd (cm/min) and the area available for ethanol transport, Al (cm^2).

Gastric emptying

Ethanol can be transported to the small intestine by convective flow through the pyloris from the stomach. The flow rate of liquids into the small intestine as a result of gastric emptying is proportional to the stomach lumen volume /15,16/. The proportionality constant which characterizes this flow is α (min^{-1}).

Simulation of ethanol pharmacokinetics with repeated dosing

Model parameters were determined by Monte Carlo simulation and chi-squared minimization as detailed earlier /14/. With the mean and standard deviation established for each model parameter, Monte Carlo simulation was again used, this time to simulate ethanol pharmacokinetics with repeated dosing. The pharmacokinetic model was solved 1,500 times for each of the 6 weeks of experimental data resulting from repeated dosing.

The structure of the model allowed for individual consideration of stomach and intestinal ethanol absorption. To estimate ethanol transport in the stomach and intestine the mass flux equations were integrated during simulation. Simulated AUC estimates were obtained by integrating the BAC versus time curve during each simulation.

Statistical analysis

The study was necessarily a repeated measures design. Therefore, repeated measures univariate ANOVA and multivariate ANOVA, using weekly dosing as the independent variable, were used for analysis of experimentally measured data and simulation data. SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULTS

Experimental ethanol pharmacokinetics with repeated dosing

Experimental mean AUC values and experimental mean -Rate values are shown in Figures 1 and 2, respectively.[1] Error bars indicate ± one standard deviation. Figure 1 indicates a slight upward trend of AUC with repeated alcohol dosing (R^2 = 0.52). Figure 2 indicates a slight upward trend for -Rate with repeated alcohol dosing (R^2 = 0.77).

Mauchly's test /17/ indicated the sphericity assumption for univariate ANOVA was valid neither for the AUC data nor the Rate data. Further, ANOVA used with the Greenhouse-Geisser degrees-of-freedom correction /18/ indicated no significant differences in the AUC mean values (p = 0.234) or the Rate mean values (p = 0.387) resulting from the weekly repeated dosing. Multivariate ANOVA also indicated no significant differences in the AUC data (F = 202.4, Wilk's Λ = 0.001, p = 0.053) or the Rate data (F = 0.579, Wilk's Λ = 0.463, p = 0.681).

It is possible that the lack of significance in the experimental AUC and Rate data was due to relatively low sample size (n = 5). This possibility was explored by repeating the statistical analysis with three sets of identical replicates of existing values from the five subjects yielding a sample size of n = 20. Multivariate ANOVA of the replicated values (n = 20) did indicate significant differences in the replicated set of experimental AUC data (Wilk's Λ= 0.001, F = 3238.4, p = 0.0) and Rate data (Wilk's Λ = 0.463, F = 5.791, p = 0.021). The observed power increased from 0.638 in the original experimental AUC data (n = 5) to 1.0 in the replicated set of experimental AUC data (n = 20). The observed power increased from 0.063 in the original experimental Rate data (n = 5) to 0.744 in the replicated set of experimental AUC data (n = 20).

[1] Elimination rate values are numerically negative and are shown in Figure 2 as -Rate values.

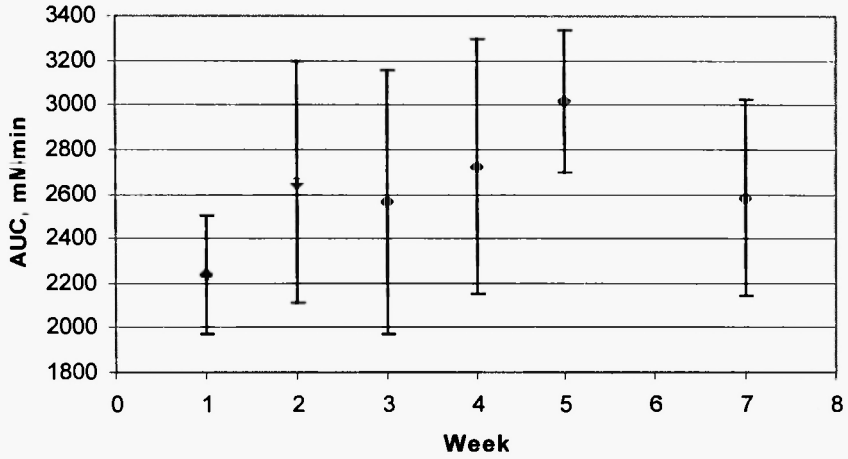

Fig. 1: Area under the blood alcohol versus time curve (AUC) with repeated weekly dosing of five dogs. Experimental AUC values (mM-min) were calculated using Simpson's rule integration of blood alcohol concentration (BAC) values. Week 6 included dosing but no blood sampling. Error bars indicate ± SD.

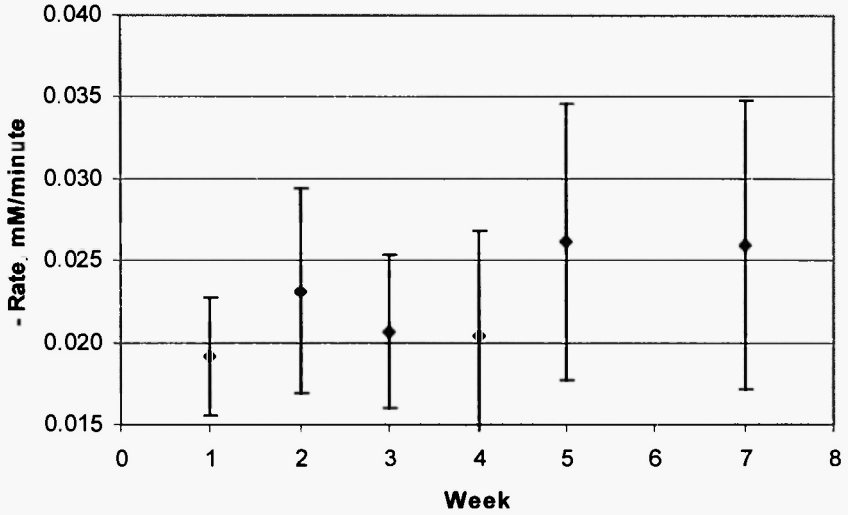

Fig. 2: Rate of ethanol disappearance from the circulation with repeated weekly dosing of five dogs. The observed elimination rate (mM/min) was the slope of the linear region of the descending portion of the BAC versus time curve determined by linear regression. Week 6 included dosing but no blood sampling. Error bars indicate ± SD.

Simulation of ethanol pharmacokinetics with repeated dosing

AUC

Mauchly's test indicated that the sphericity assumption for univariate ANOVA was not valid for the simulated AUC values. However, application of the Greenhouse-Geisser correction (ε = 0.838) with ANOVA did indicate significant differences in the simulated AUC mean values ($F = 970.4$, $p = 0.0$). Multivariate ANOVA also indicated significant differences in the simulated AUC data (Wilk's $\Lambda = 0.166$, $F = 1564.6$, $p = 0.0$).

Ethanol absorption

Simulation allowed computation of cumulative ethanol flux into the circulation from the stomach and the small intestine (Fig. 3). Figure 3 shows that ethanol absorbed in the stomach decreased significantly (Wilk's $\Lambda = 0.096$, $F = 2911.0$, $p = 0.0$) with repeated ethanol dosing, and ethanol absorbed in the small intestine increased significantly (Wilk's $\Lambda = 0.169$, $F = 1527.7$, $p = 0.0$) with repeated ethanol dosing.

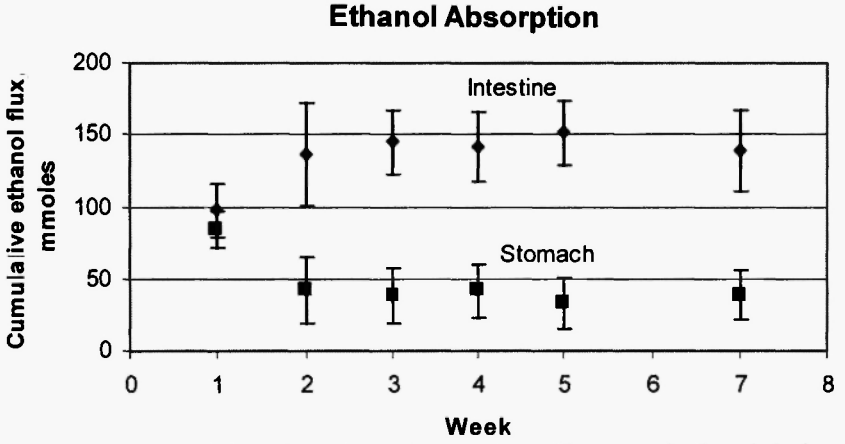

Fig. 3: Apparent habituation of gastric emptying with repeated ethanol dosing. Ethanol absorption shifted from approximately equal absorption in the stomach and intestine to three-fold more absorption in the intestine than in the stomach. This shift is significant since ethanol absorption in the small intestine is six-fold more rapid than ethanol absorption in the stomach.

DISCUSSION

Ostensibly, increasing AUC values (Fig. 1) could possibly indicate either a slower rate of ethanol elimination from the circulation due to metabolism, or the movement of ethanol from some other compartment into the circulation, or both. Similarly, the -Rate trend (Fig. 2) could possibly indicate a faster rate of ethanol elimination from the circulation due to metabolism, or the accelerated movement of ethanol from the circulation into some other compartment, or both. Clearly, the metabolic rate cannot simultaneously increase to accelerate the rate of ethanol elimination (-Rate) and also decrease to increase AUC values.

In this study -Rate was determined empirically by observing ethanol elimination from the circulation. However, mean weekly −Rate values were not correlated (R^2 = 0.095) with the mean weekly V_{max} values obtained by fitting data to the model for the cohort, even though -Rate is indeed a deterministic function of V_{max}: if V_{max} was zero then -Rate would also be zero. -Rate can also be influenced by ethanol absorption. -Rate correlated negatively with cumulative intestinal ethanol absorption (R^2 = -0.59) and correlated positively with cumulative gastric ethanol absorption (R^2 = 0.64). Ethanol elimination from the circulation can only occur when ethanol is present in the circulation. It is apparent from Figure 3 that with repeated ethanol dosing relatively more ethanol was absorbed in the small intestine than in the stomach. Since ethanol absorption from the intestine is more than six-fold more rapid than ethanol absorption from the stomach /14/, as relatively more ethanol was absorbed in the intestine with repeated ethanol dosing, the observed elimination rate increased since more ethanol was available for metabolism in the circulation.

Increasing cumulative intestinal ethanol absorption and decreasing cumulative gastric ethanol absorption were responsible for the increase in AUC with repeated ethanol dosing. AUC correlated positively with cumulative intestinal ethanol absorption (R^2 = 0.86) and correlated negatively with cumulative gastric ethanol absorption (R^2 = -0.82). Since intestinal ethanol absorption allowed relatively more rapid ethanol entry into the circulation, ethanol was therefore present in the circulation sooner and in relatively greater amounts than when gastric ethanol absorption was nearly equal to intestinal absorption, thus increasing AUC values.

Finally, during 7 weeks of repeated ethanol dosing, ethanol absorption shifted from approximately equal absorption in the stomach and intestine to three-fold more absorption in the intestine than in the stomach. This finding was unexpected. The likely cause of the shift in absorption site was probably a concomitant change in gastric emptying that occurred with repeated dosing. Although the proportionality constant, α, was not correlated with repeated ethanol dosing (R^2 = 0.032), it was correlated negatively with gastric ethanol absorption (R^2 = -0.579) and positively with intestinal ethanol absorption (R^2 = 0.534).

Because this was a repeated measures design no ethanol-free control group was available. However, since water was available to the dogs *ad libitum* except during the experiment it seems unlikely that water in the gavage caused the shift in absorption site. In a human study using a single acute dose delivered via intubation, 5-40% w/v ethanol gavage delayed gastric emptying compared with water gavage /19/. Other studies demonstrated that gastric emptying delay increases as the ethanol concentration in the dose increases /20/. However, it is important to differentiate between ethanol's acute delay of gastric emptying found in previous studies and the finding reported here. In the present study, starting with naive dogs, during repeated weekly dosing, gastric emptying in the presence of ethanol apparently became habituated to the presence of ethanol: with each repeated dose, gastric emptying apparently changed, thereby shifting ethanol absorption from the stomach to the intestine where ethanol absorption is six-fold more rapid than in the stomach. Additional research will be required to explore the nature of this apparent habituation.

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