Between-subject and within-subject variations in the pharmacokinetics of ethanol

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- 1 Twelve healthy men drank 0.80 g ethanol kg⁻¹ body weight on four occasions spread over several weeks. Ethanol was given as 96% v/v solvent which was diluted with orange juice to make a cocktail (20–25% v/v). This drink was ingested in exactly 30 min at 08.00 h after an overnight (10 h) fast.
- 2 Samples of venous blood were obtained at exactly timed intervals of 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after the start of drinking. The concentrations of ethanol in whole blood were determined by headspace gas chromatography.
- 3 Summary measures were used to evaluate the concentration-time profiles of ethanol for each subject. The between-subject and within-subject components of variation for the pharmacokinetics of ethanol were derived by one-way analysis of variance (ANOVA).
- 4 The variation between different subjects dominated the total variance for all of the pharmacokinetic parameters studied except the rate of disappearance of ethanol from blood (k_o). For this latter parameter, 42% and 58% of the total variation arose from variations between- and within-subjects respectively. These results might be important to consider when experiments on the clinical pharmacokinetics of ethanol are being planned.

Keywords alcohol pharmacokinetics variability reproducibility ANOVA variance components

Introduction

We recently investigated whether certain drugs used to suppress the production of gastric acid (cimetidine, ranitidine and omeprazole) could modify the pharmacokinetics of ethanol [1]. None of these agents influenced the absorption, distribution, or elimination kinetics of ethanol when compared with a no-drug, control treatment. Our experimental protocol involved a four-part crossover design and the ethanol was ingested on an empty stomach. The lack of any treatment effects and the fact that the same dose of ethanol (0.8 g kg⁻¹) was administered to the same individual on four occasions, allowed us to calculate the magnitude of between-subject and within-subject variation in the pharmacokinetics of ethanol.

Methods

Subjects and protocol

Twelve healthy male volunteers gave written informed consent to participate in these experiments. Their mean age was 27.3 years (s.d. 4.8), mean body weight was 75.2 kg (s.d. 3.9) and mean height was 181 cm (s.d. 4.1). All subjects were accustomed to moderate drinking and some were smokers but they were not allowed to smoke during the experiments. The study protocol was approved by the ethics review board at the University Hospital in Linköping.

Each volunteer participated in four drinking experiments in randomized order separated by 1 to 4 weeks. They arrived at the hospital at about 07.00 h

after an overnight (10 h) fast and swallowed a tablet of either cimetidine (800 mg Tagamet®), ranitidine (300 mg Zantac®) or omeprazole (20 mg Losec®) and 1 h later drank 0.8 g ethanol kg⁻¹ body weight in exactly 30 min. The same drugs had been taken daily for 7 days before the ingestion of ethanol. The alcoholic drink was made from 96% v/v ethanol solvent which was diluted to 20–25% v/v with orange juice. A lunch was served 4 h after the start of drinking.

Blood sampling and assay of ethanol

Samples of venous blood were obtained through an indwelling catheter at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min, timed from the start of drinking. Blood samples were collected into 5 ml Vacutainer® tubes (Becton Dickenson, USA) containing 20 mg sodium fluoride and 143 units of sodium heparin. The samples were stored at 4° C until assayed the next day by headspace gas chromatography as described by Jones & Schuberth [2]. Aliquots of whole blood were removed in duplicate from the Vacutainer® tubes and diluted 11 times with *n*-propanol (8 mg dl⁻¹ in water) as the internal standard. The assay was linear over a wide range of concentration (0–300 mg dl⁻¹) and the calibration line (peak height ratio vs concentration of ethanol) passed through the origin. The standard deviation (s.d.) of the assay increased with the blood concentration of ethanol. At a mean concentration of 80 mg dl⁻¹, the s.d. was 1 mg dl⁻¹; the limit of detection was less than 1 mg dl⁻¹.

Pharmacokinetic analysis

The concentrations of ethanol were plotted for each subject and the rate of ethanol disappearance from blood was determined as described by Widmark [3]. Zero-order elimination was assumed in the post-absorptive phase until the blood alcohol concentration had decreased to 5–10 mg dl⁻¹. Values of $C_{\max}$, and $t_{\max}$ were noted for each subject and the ratio $C_{\max}/t_{\max}$ was used as an indication of the rate of absorption of ethanol. The slope of the rectilinear elimination phase (k_o) was determined by linear regression. The number of data points included in this analysis differed among subjects depending on when $C_{\max}$ was achieved. The y-intercept ($C(0)$) and the x-intercept (time_o) were derived from the regression equation. The ratio of dose of ethanol (g kg⁻¹) to $C(0)$ provided an estimate of the apparent volume of distribution of ethanol (V) in units of l kg⁻¹. The ratio of alcohol dose (g kg⁻¹) to time_o gave an indication of the overall rate of disposal of ethanol from the body, having units of g kg⁻¹ h⁻¹. Values of AUC(0,6 h) were estimated using the linear trapezoidal rule.

Statistical analysis

Means, standard deviations (s.d.), coefficients of variation (CV), and linear regression equations were calculated by standard methods. The components of variation between and within subjects were calculated

using model II (random effects) one-way analysis of variance (ANOVA) as described by Snedecor & Cochran [4]. Rodbard [5] presents a particularly lucid account of the calculation of variance components. The 12 subjects were considered to be a random sample selected from a population of similar subjects and the 4 drinking sessions were considered to provide replicate measurements of the pharmacokinetics of ethanol in each subject.

Results

Figure 1 shows the concentrations of ethanol during the four drinking experiments in each of the 12 subjects. These data indicate a high degree of within-subject reproducibility in ethanol kinetics. Table 1 gives details of the absorption kinetics of ethanol. The values of $C_{\max}$ and median times to reach $t_{\max}$ as well as the rate of absorption [$C_{\max}/t_{\max}$] showed good repeatability in the four drinking experiments. Table 2 illustrates the method of calculating components of variance for between- and within-subject variations in k_o by one-way ANOVA. Table 3 summarises the results of ANOVA for the other kinetic parameters. Because $t_{\max}$ is a discrete variable ANOVA was not applied to these data. However, there was clearly more variation in the time to reach $C_{\max}$ between subjects than within subjects. *F*-ratios confirmed significantly more variation between than within subjects for all of the pharmacokinetic parameters.

Discussion

Inter- and intra-individual variation in pharmacokinetics needs to be considered when planning experiments in clinical pharmacology [6, 7]. Whether an experimental design should include two parallel groups (between subjects) or a two-part crossover design (within subjects) might depend on the relative magnitude of the between- and within-subject components of variation [6, 8]. Furthermore, inherent variability in the pharmacokinetics of ethanol should be considered when expert statements are made in forensic medicine and toxicology, e.g. concerning driving under the influence of alcohol [9–11]. To determine the relative influences of genetic and environmental factors on the metabolism of ethanol, Vesell *et al.* [12] studied its rate of disappearance from blood (k_o) in monozygotic and dizygotic twins. A strong genetic component in the rate of ethanol metabolism was reported but their results were difficult to rationalise because of an unusually large difference in the apparent volume of distribution of ethanol in the dizygotic twins. Indeed, other workers have failed to confirm such a clearcut genetic influence on the rate of elimination of ethanol in monozygotic and dizygotic twins [13, 14].

Figure 1 Blood concentrations of ethanol in 12 healthy men after ingestion of 0.8 g ethanol kg⁻¹ body weight on an empty stomach over 30 min on four separate occasions.

Table 1 Indirect indices of ethanol absorption rate (trial 1 = control, no drug; trial 2 = cimetidine; trial 3 = ranitidine; trial 4 = omeprazole)

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
$C_{\max}$ (mg dl ⁻¹)*	105 (79–144)	101 (86–118)	103 (86–145)	102 (76–144)
$t_{\max}$ (min) [†]	60 (30–120)	60 (45–120)	63 (45–90)	60 (30–120)
$[C_{\max}/t_{\max}]^{\dagger}$ (mg dl ⁻¹ min ⁻¹)	1.89 (0.53–4.8)	1.53 (0.73–2.6)	1.83 (0.96–3.2)	1.76 (0.86–4.8)

*Mean and range. [†]Median and range.

Table 2 Results of one-way ANOVA used to calculate between-subject and within-subject components of variance for the rate of disappearance of ethanol from venous blood (k_o)

Source of variation	DF ¹	Sum of squares	Mean square (MS)	F ratio
Between subjects (b)	11	56.32	5.12	3.93
Within subjects (w)	36	46.94	1.30	
Total	47	103.26		

¹Degrees of freedom.

Variance component between subjects (V_b):

$V_b = (MS_b - MS_w)/4 = 0.954$, $SD_b = 0.976$, $CV_b = 6.9\%$.

Variance component within subjects (V_w):

$V_w = 1.30$, $SD_w = 1.14$, $CV_b = 8.0\%$.

The total variance for a single measurement of k_o is given by the sum of variance between (V_b) and variance within (V_w) = 2.258.

Between subject variation (%) = $[0.954/2.258] \times 100 = 42\%$.

Within subject variation (%) = $[1.30/2.258] \times 100 = 58\%$.

Many studies have considered inter-individual variability in the kinetics of ethanol [10, 15]. Reported coefficients of variation for $C_{\max}$ and k_o range from 10% to 20%. By contrast, much less attention has been given to the magnitude of variation within the same subject despite the importance of such information in experimental design and pharmacokinetics [16]. Schönheyder *et al.* [17] found that the within-subject coefficients of variation of k_o and V were 8% and 10%, respectively, based on experiments with three male subjects who drank 40 g ethanol on ten separate occasions. The variability between- and within-subjects for these kinetic parameters were similar. Passananti *et al.* [18] studied 10 male subjects who drank 0.79 g ethanol kg⁻¹ on four occasions and measured the blood alcohol concentration for 4 h during the post-absorptive phase. The average within-subject coefficient of variation for k_o was 8% with a range of 3% to 10%. The corresponding between-subject variation was 14% on average with a

Table 3 Between-subject (SD_b) and within-subject (SD_w) components of variation for the pharmacokinetics of ethanol derived by one-way ANOVA. The percentage of the total variation between and within subjects is shown in brackets

Parameter	Mean $\pm$ s.d. ¹	Components of variation		Variance ratios (F)
		SD_b (%) ²	SD_w (%) ³	
C_{max} (mg dl ⁻¹)	103 $\pm$ 16	14.13 (75%)	8.18 (25%)	12.9
k_o (mg dl ⁻¹ h ⁻¹)	14.2 $\pm$ 1.5	0.98 (42%)	1.14 (58%)	3.93
$C(0)$ (mg dl ⁻¹)	117 $\pm$ 9	8.12 (75%)	4.74 (25%)	12.7
Time _o (h)	8.3 $\pm$ 0.8	0.68 (66%)	0.484 (34%)	8.89
Elimination rate (mg kg ⁻¹ h ⁻¹)	97.2 $\pm$ 10.1	8.41 (68%)	5.82 (32%)	9.35
V (l kg ⁻¹)	0.69 $\pm$ 0.05	0.046 (73%)	0.028 (27%)	11.89
$C(4\text{ h})$ (mg dl ⁻¹)	63 $\pm$ 8	7.46 (78%)	3.99 (22%)	14.96
AUC (mg dl ⁻¹ h)	393 $\pm$ 52	50.5 (89%)	18.1 (11%)	32.80

¹s.d. based on all 48 individual measurements and reflecting between- and within-subject variability.

² SD_b is the standard deviation between subjects if an infinite number of tests had been made on each.

³ SD_w is the standard deviation within subjects and applies to a single measurement for a new subject from the same population.

F ratios for 11 and 36 degrees of freedom; $F_{p=0.05} = 2.07$;

$F_{p=0.001} = 3.86$. The inter-individual variability was significantly greater than intra-individual variability for all parameters.

range from 10% to 17%. None of these previous studies has divided the total variance into its within- and between-subject components using appropriate statistical methods. Moreover, our study also considered the absorption kinetics of ethanol and its variability between- and within-subjects.

Our results speak against the use of parallel group designs when the disposition of ethanol and its bio-availability are of interest. This follows from the larger contribution of between-subject variation to total variation with respect to AUC, V , and $C(0)$. The large between-subject variation in these parameters (73–89%) is probably explained by differences in body composition. We administered alcohol per kg of body weight and individual differences in the ratio of fat/lean tissue may have influenced the between-subject variation. Most (75%) of the total variation in C_{max} was attributed to inter-individual differences, and this probably reflects variation in stomach emptying times [19]. The value of C_{max} was inversely related to t_{max} ($r = -0.54$, $P < 0.001$) which indicates that slow absorbers of alcohol reached lower peak BAC. With reference to the rate of disposal of ethanol, as reflected in time_o and the elimination rate (mg kg⁻¹ h⁻¹), about two-thirds of

the total variance (66% and 68%, respectively) arose from variation between subjects. This conflicts with the disappearance rate of ethanol from blood (k_o) where 42% and 58% of total variation was associated with inter- and intra-individual variation, respectively. However, the slope of the rectilinear elimination phase (k_o), depends not only on the activity of alcohol metabolising enzymes but also on the volume of distribution of ethanol. The smaller the value of V the steeper the slope of the elimination phase (k_o) [15].

Our original experiment [1] failed to show any statistically significant effect of the different drug treatments on ethanol kinetics. However, if such an effect was actually present but remained undetected, e.g. because of the relatively small sample sizes, the magnitude of within-subject variation reported here might be exaggerated.

We conclude that, for studies aimed at comparing the influence of various treatments on the disappearance rate of ethanol from blood, a parallel group design may prove as effective as a crossover design. However, if factors influencing the systemic availability of ethanol are of concern then a crossover design is preferable.

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