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BLOOD ALCOHOL LEVELS IN RATS: NON-UNIFORM YIELDS FROM INTRAPERITONEAL DOSES BASED ON BODY WEIGHT

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1 Sprague-Dawley rats ($n = 72$) weighing from 125 to 450 g were injected intraperitoneally (i.p.) with 16% (w/v) ethanol to provide 1, 2, or 3 g/kg doses.

2 Resulting blood alcohol levels (BALs) demonstrated a general inadequacy of dose/body weight (g/kg) formulations of ethanol to provide uniform BALs in animals of different weights.

3 BAL differences between heavier and lighter rats were not well accounted for by developmental changes in liver weight or alcohol dehydrogenase activity.

4 From the data, a table was derived of more appropriate ethanol injection volumes to produce 0–300 mg% BALs (20 mg% increments) in rats from 100–500 g (10 g increments).

Introduction

In studies of the acute effects of ethanol, an obvious and important variable to be controlled is the ethanol dose. The dose/body weight formulation appears to be an almost universally accepted means for accomplishing uniform ethanol dosage. Ideally, the adequacy of such a formulation will be checked by measuring blood alcohol levels (BALs) directly. However, BALs are not always given.

The purpose of the present experiments was to verify our anecdotal observations that dose/body weight formulations are as inadequate in providing uniform BALs among different weight rats as formulations which do not correct for weight at all. The primary difference is that a linear correction for weight (as in the g/kg formulation) overestimates dosages, while a failure to correct for weight underestimates them. In either case, the degree of error is approximately the same, though opposite in direction. We also examined parameters of liver ethanol metabolism in rats of different ages and weights. From our BAL measurements, we developed a table which gives intraperitoneal injection volumes of 16% (w/v) ethanol required to achieve predictable BALs from 0 to 300 mg% in rats weighing from 100 to 500 g.

Methods

A total of 72 naïve male Sprague-Dawley rats (Charles River Farms, Wilmington, Delaware) were divided into 7 different weight groups (mean weights of 135, 182, 247, 286, 311, 357, and 419 g). Weights within each group were relatively uniform, with s.e.means ranging from ±1 to ±7 g. Randomly assigned rats within each weight group were then injected intraperitoneally with either 1, 2, or 3 g/kg ethanol using a 16% (w/v) solution.

Tail vein blood samples from each rat were taken at 10, 60, and 120 min post-injection. Following the 120 min blood sample, rats were decapitated, whole livers dissected, weighed, perfused with Ringer solution and frozen in liquid nitrogen for later analysis. BALs were assayed using the Sigma NAD-ADH multi-test kit No. 331–10. Liver alcohol dehydrogenase (ADH) activity was measured according to the procedure of Markovic, Theorell & Rao (1971).

The BAL data were analysed by Pearson Product-Momentum correlation statistics and by standard 3-way repeated measures analysis of variance (ANOVA) techniques. For the ANOVA, the first factor was weight group, the second factor was ethanol dose (g/kg), and the third, repeated measures factor was BAL (mg%) at 10, 60, and 120 min post-injection. The liver data were assessed by a two-way ANOVA with weight group as the first factor, and ethanol dose (g/kg) as the second factor. P values of less than 0.05 were considered significant.

Results

Ethanol concentrations and metabolism

As expected, giving 1, 2, or 3 g/kg ethanol produces significantly different BALs ($P < 0.001$). There is only a small, but significant, effect on BALs of time after injection (ethanol metabolism) ($P < 0.05$) (Figure 1a). BALs generally peak within 10 min and remain stable for at least 50 min thereafter. At 2 and 3 g/kg, BALs are relatively stable for even longer post-injection periods from 10 to 120 min after in-

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traperitoneal administration (Figure 1a). Thus, the significant effect on BALs of post-injection time derives almost wholly from the 120 min decrease in mean BAL of rats receiving the 1 g/kg dose. This conclusion is further supported by the significant interaction of ethanol metabolism with ethanol dose (i.e., the rate of ethanol clearance is not uniform for the three drug doses) ($P<0.001$). Finally, there is a significant interaction of ethanol metabolism and rat weight ($P<0.001$). Inspection of the data indicates that ethanol clearance is faster in ligher animals. However, this may simply reflect saturation of clearance mechanisms at the higher BALs uniformly shown by heavier animals (see below).

BALs resulting from 1, 2, and 3 g/kg ethanol doses differ significantly depending upon body weight ($P<0.001$). Lighter rats consistently exhibit lower BALs with the same g/kg dose than their heavier cohorts. Moreover, inspection of the data and a significant interaction of ethanol dose and weight group ($P<0.01$) suggest that the effect of weight on BALs is particularly marked at lower g/kg ethanol doses. When mean BALs after the various ethanol doses are expressed as a function of mean body weight of the different groups (Figure 1b), the g/kg formulation is seen to produce uniform BALs only in the range usually associated with coma or death (300 mg% or more); the dose/body weight (g/kg) formulation does not produce uniform BALs in the range associated with ataxia (100–300 mg%) or sedation (100 mg% or less).

Because there were 7 different weight groups and 3 different g/kg ethanol doses, injection volumes to achieve a high g/kg dose in lighter weight rats sometimes overlapped injection volumes to achieve a lower g/kg dose in heavier rats. For example, the 2 g/kg injection volume for 138 g rats (1.73 ml) is comparable to the 1 g/kg injection volume for 281 g rats (1.76 ml). Thus, the resultant mean BALs for these two groups provide an estimate of the effect of not correcting for subject weight. Based on such comparisons, giving similar doses in comparable injection volumes without correcting for subject weight results in an average underdose in the heavier animal of approximately 60%, while giving different doses on a g/kg basis to correct for the weight of the animal results in an average overdose of approximately 50%.

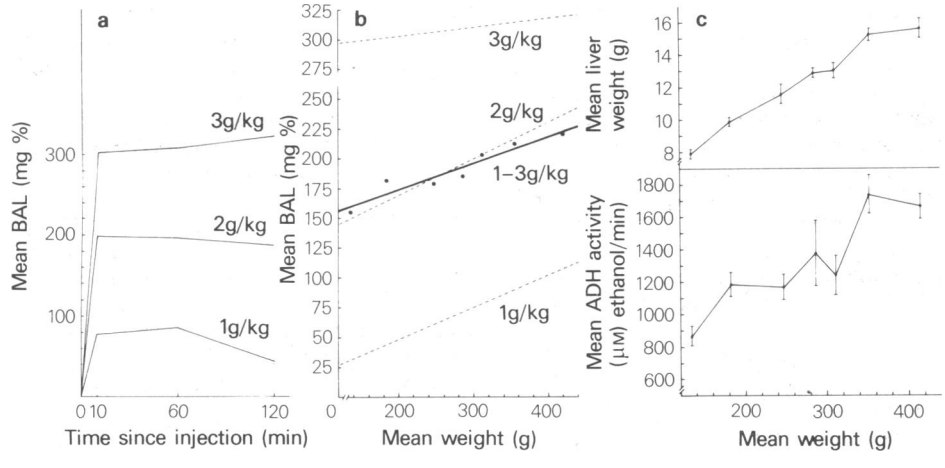

Figure 1 (a) Time course of blood ethanol clearance after 1, 2, and 3 g/kg ethanol doses. Each data point represents the mean blood alcohol level (BAL) at a particular dose averaged over the seven weight groups to summarize the overall effects of time since injection. Near peak BALs are reached within 10 min of intraperitoneal injection, with BALs relatively stable for the ensuing 50 min. There is clear evidence of ethanol clearance only at the lowest dose from 60–120 min after administration. (b) BAL as a function of subject weight and g/kg ethanol dose. Each data point represents the mean BAL of a particular weight group averaged over 1, 2 and 3 g/kg ethanol doses and 10, 60, and 120 min samples. Correlation coefficient of the regression (solid) line is $r=0.95$ ($P<0.001$). Results for individual ethanol doses are shown by dashed lines; correlation coefficients for the 1, 2, and 3 g/kg regression lines are $r=0.78$ ($P<0.001$), $r=0.86$ ($P<0.001$), and $r=0.30$ ($P<0.05$), respectively. The relatively gentle slope of the 3 g/kg regression line suggests that at very high doses a linear correction for body weight (i.e., g/kg) may be appropriate. (c) Developmental changes in liver weight (above) and ADH activity (below). Note that the approximately 3 fold increase in body weight from 36 to 100 days of age is accompanied by a less than 2 fold increase in liver weight and liver ADH activity.

Liver metabolism of ethanol

There is a significant developmental increase in liver weight from 36 to 100 days of age (Weight Group 1 to Weight Group 7) ($P<0.001$). This increase is accompanied by a significant increase in ADH activity per liver ($P<0.001$) (Figure 1c). Note, however that developmental increases in liver ADH activity do not keep pace with developmental increases in body weight (i.e., for every 100% increase in body weight there is a corresponding ADH increase of only approximately 34%).

Discussion

The results presented above indicate the inadequacy of g/kg formulations for producing uniform BALs among different animals. Moreover, the data can be used to generate a table for more accurate intraperitoneal ethanol formulation. Table 1 provides ml doses of 16% (w/v) ethanol necessary to achieve BALs from 0–300 mg% in rats weighing from 100–500 g. The volumes given are derived from interpolations of the slopes and Y-intercepts of the regression lines in Figure 1b. Subsequent tests of the

Table 1 Ethanol dosages (ml of 16% w/v ethanol in saline) to produce blood ethanol levels from 0–300 mg% in Sprague-Dawley rats weighing from 100–500 g

	Desired blood ethanol level (mg%)															
Rat wt. (g)	0	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300
100	0.00	0.45	0.66	0.77	0.88	0.98	1.09	1.20	1.28	1.36	1.44	1.52	1.60	1.68	1.76	1.84
110	0.00	0.45	0.71	0.83	0.95	1.06	1.18	1.30	1.40	1.49	1.58	1.67	1.76	1.85	1.94	2.03
120	0.00	0.45	0.76	0.89	1.03	1.15	1.28	1.41	1.52	1.62	1.72	1.82	1.92	2.02	2.12	2.22
130	0.00	0.45	0.82	0.96	1.10	1.23	1.34	1.52	1.63	1.74	1.85	1.96	2.08	2.19	2.30	2.41
140	0.00	0.45	0.87	1.01	1.16	1.31	1.46	1.61	1.75	1.87	1.99	2.11	2.23	2.35	2.47	2.59
150	0.00	0.45	0.91	1.07	1.23	1.38	1.54	1.70	1.86	1.99	2.12	2.25	2.38	2.51	2.64	2.77
160	0.00	0.45	0.93	1.12	1.29	1.45	1.63	1.80	1.97	2.11	2.25	2.39	2.53	2.67	2.82	2.96
170	0.00	0.45	0.93	1.17	1.35	1.52	1.71	1.89	2.07	2.22	2.37	2.53	2.68	2.83	2.99	3.14
180	0.00	0.48	0.93	1.22	1.41	1.59	1.78	1.97	2.16	2.33	2.50	2.66	2.83	2.99	3.16	3.32
190	0.00	0.48	0.93	1.27	1.47	1.66	1.86	2.06	2.26	2.44	2.62	2.80	2.97	3.15	3.32	3.50
200	0.00	0.48	0.95	1.34	1.58	1.79	2.00	2.21	2.42	2.62	2.81	2.99	3.18	3.37	3.56	3.75
210	0.00	0.48	0.95	1.38	1.63	1.85	2.07	2.29	2.50	2.72	2.92	3.12	3.32	3.52	3.73	3.93
220	0.00	0.48	0.95	1.42	1.68	1.91	2.13	2.36	2.59	2.81	3.03	3.24	3.46	3.67	3.89	4.10
230	0.00	0.48	0.95	1.46	1.72	1.96	2.20	2.43	2.67	2.91	3.14	3.37	3.59	3.82	4.05	4.28
240	0.00	0.48	0.95	1.50	1.77	2.01	2.26	2.50	2.75	3.00	3.24	3.48	3.72	3.97	4.21	4.45
250	0.00	0.48	0.95	1.50	1.81	2.06	2.32	2.57	2.83	3.08	3.34	3.60	3.85	4.11	4.37	4.62
260	0.00	0.48	0.98	1.50	1.84	2.11	2.37	2.64	2.90	3.16	3.43	3.71	3.98	4.25	4.52	4.80
270	0.00	0.50	0.98	1.50	1.88	2.15	2.43	2.70	2.97	3.24	3.52	3.81	4.10	4.39	4.68	4.96
280	0.00	0.50	0.98	1.50	1.91	2.19	2.48	2.76	3.04	3.32	3.61	3.91	4.22	4.52	4.83	5.13
290	0.00	0.50	0.98	1.52	1.94	2.23	2.53	2.82	3.11	3.40	3.69	4.01	4.33	4.65	4.97	5.30
300	0.00	0.50	0.98	1.52	1.97	2.27	2.57	2.87	3.17	3.47	3.77	4.10	4.44	4.78	5.12	5.46
310	0.00	0.50	0.98	1.52	2.00	2.31	2.62	2.92	3.23	3.54	3.85	4.19	4.55	4.91	5.26	5.62
320	0.00	0.50	0.98	1.52	2.03	2.34	2.66	2.97	3.29	3.61	3.92	4.27	4.65	5.03	5.40	5.78
330	0.00	0.50	0.98	1.52	2.03	2.37	2.70	3.02	3.35	3.67	3.99	4.35	4.75	5.14	5.54	5.93
340	0.00	0.50	0.98	1.52	2.03	2.40	2.73	3.07	3.40	3.73	4.06	4.42	4.84	5.25	5.67	6.09
350	0.00	0.50	0.98	1.52	2.03	2.43	2.77	3.11	3.45	3.79	4.13	4.49	4.92	5.36	5.80	6.24
360	0.00	0.50	1.01	1.52	2.03	2.45	2.80	3.15	3.55	3.90	4.25	4.60	5.05	5.52	5.98	6.44
370	0.00	0.51	1.01	1.52	2.03	2.48	2.83	3.19	3.60	3.95	4.31	4.67	5.13	5.61	6.10	6.58
380	0.00	0.51	1.01	1.52	2.03	2.50	2.86	3.23	3.64	4.01	4.37	4.74	5.20	5.71	6.22	6.73
390	0.00	0.51	1.01	1.52	2.03	2.51	2.89	3.26	3.68	4.06	4.43	4.80	5.26	5.79	6.33	6.86
400	0.00	0.51	1.01	1.52	2.03	2.53	2.91	3.29	3.72	4.10	4.49	4.87	5.31	5.87	6.44	7.00
410	0.00	0.51	1.01	1.52	2.03	2.53	2.93	3.32	3.76	4.15	4.54	4.93	5.36	5.95	6.54	7.13
420	0.00	0.51	1.01	1.52	2.07	2.53	2.96	3.35	3.80	4.19	4.59	4.99	5.39	6.01	6.63	7.25
430	0.00	0.51	1.01	1.52	2.07	2.53	2.97	3.38	3.83	4.24	4.64	5.04	5.45	6.07	6.72	7.38
440	0.00	0.51	1.01	1.52	2.07	2.53	2.99	3.40	3.86	4.27	4.69	5.10	5.51	6.12	6.80	7.49
450	0.00	0.55	1.05	1.56	2.07	2.53	3.01	3.42	3.89	4.31	4.73	5.15	5.57	6.16	6.88	7.60
460	0.00	0.55	1.05	1.56	2.07	2.53	3.02	3.44	3.92	4.35	4.77	5.20	5.63	6.19	6.95	7.71
470	0.00	0.55	1.05	1.56	2.07	2.53	3.03	3.46	3.95	4.38	4.81	5.25	5.68	6.21	7.01	7.81
480	0.00	0.55	1.05	1.56	2.07	2.53	3.04	3.48	3.97	4.41	4.85	5.29	5.73	6.22	7.06	7.90
490	0.00	0.55	1.05	1.56	2.07	2.53	3.04	3.49	3.99	4.44	4.89	5.34	5.78	6.23	7.10	7.98
500	0.00	0.55	1.05	1.56	2.07	2.53	3.04	3.51	4.01	4.47	4.92	5.38	5.83	6.29	7.12	8.05

values given in Table 1 show it to be reliable, with the average deviation of predicted BALs, relative to actual BALs, less than ±9 mg% (range tested = 80–260 mg% in rats from 170–420 g, $n = 24$). The BALs obtained should be relatively stable from 10 to 60 min post-injection. Our data (Figure 1a) also indicate that very large injection volumes may result in stable BALs for as long as 120 min. This is probably due to the fact that only a portion of a large intraperitoneal injection can be immediately absorbed; continuing ethanol uptake by the gut provides a counterbalance to the normal 40–60 mg% per hour catabolism of the substance, and thus BALs do not appear to decline for several hours after injection.

There are many possible bases for the general failure of g/kg intraperitoneal ethanol to result in uniform BALs among different weight subjects. For example, the relationship between the peritoneal absorption area and the injection volume may vary developmentally. The inability of liver ADH activity to keep pace with developmental increases in body weight also seems a likely alternative. However, the variability associated with g/kg ethanol doses in different weight rats is as apparent 10 min after injection as 120 min after injection. Since the rate of ethanol clearance by the liver is only 40–60 mg% per hour, or 7–10 mg% for any 10 min epoch, the contribution of liver metabolism to the variation in 10 min BALs must be relatively trivial. Nevertheless, the relatively greater ADH activity/liver in smaller animals may play a role in the increased rate of clearance at later time intervals (e.g. 120 min) noted in this study.

The g/kg formulation assumes that the relative size of various compartments for ethanol absorption remains constant over age-dependent increases in body weight. For the rat, this is not likely to be the case: there is a disproportionate increase in body fat with age (Lesser, Deutsch & Markofsky, 1973). Since ethanol is almost insoluble in tissue lipids compared to tissue water (Harger & Halpien, 1956), the g/kg formulation would appear to be demanding greater amounts of ethanol to fill a relatively insoluble compartment. Moreover, Wiberg, Samson, Maxwell, Coldwell & Trenholm (1971) have shown that differences in ethanol toxicity between 3 month and 12 month old rats are well accounted for by the relatively greater percentage of body water in younger rats. Other experiments of a more specific pharmacokinetic nature will be necessary to characterize the compartmentalization of ethanol in various body tissues and the physiological basis for appropriate ethanol formulation.

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