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ETHANOL METABOLISM IN HEAVY DRINKERS AFTER MASSIVE AND MODERATE ALCOHOL INTAKE

SUSANNE KEIDING,* NIELS J. CHRISTENSEN,† STIG E. DAMGAARD,‡ ANDERS DEJGÅRD,* HENNING L. IVERSEN,‡ ANNY JACOBSEN,§ SØREN JOHANSEN,‖ FRANK LUNDQUIST,‡ ELISABETH RUBINSTEIN* and KJELD WINKLER*

* Division of Hepatology, Medical Department and Department of Clinical Physiology, Hvidovre Hospital, University of Copenhagen; † Department of Internal Medicine and Endocrinology, Herlev Hospital, University of Copenhagen; ‡ Department of Biochemistry A, University of Copenhagen; § Out-patient Clinic for Alcoholics, Forchhammersvej; ‖ Institute of Mathematical Statistics, University of Copenhagen, Copenhagen, Denmark

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Abstract Some alcoholics have a regular daily alcohol consumption of more than 100 g. In preliminary observations we had the impression that the claimed alcohol intake in such 'heavy drinkers' was higher than could be accounted for by the ethanol elimination rate as measured routinely at 10 mmol/l (0.5 g/l). We therefore measured the ethanol elimination rate at very high blood ethanol concentrations of 40–80 mmol/l (2–4 g/l) found in eight alcoholics following heavy alcohol intake by measuring the falling blood ethanol concentrations until being less than 1 mmol/l. The elimination rate, on average 83 μmol/min per l blood, was about 49% higher than the elimination rate measured at 10 mmol/l in the same subject, being on average 58 μmol/min per l/blood (paired *t*-test, $P < 0.05$). The elimination rate following the high initial ethanol concentrations remained high until the concentration was below 5 mmol/l.

Calculations of elimination rates are based on a number of assumptions concerning the physiologic and metabolic conditions. We examined specifically if the concentration–time curves could be adequately described by assuming metabolism according to a Michaelis–Menten pathway with a low K_m value (simulating alcoholdehydrogenase with K_m 0.2 mmol/l) or by assuming metabolism by two pathways with an alternative high-K_m pathway with K_m about 10 mmol/l. It was not necessary, in the statistical analysis, to include an alternative high-K_m pathway. On the other hand, the data does give room for up to 50% elimination via such alternative pathways.

The elimination rate at the high concentrations corresponded roughly to the claimed daily alcohol intake; furthermore the measured elimination rate at the lower concentrations were similar to values in non-alcoholics.

Some alcoholics have a daily alcohol consumption of at least 100 g ('heavy drinkers'). It is a common observation that such subjects often claim a regular intake of alcohol which is considerably higher than can be accounted for by the usually accepted measurement of the elimination rate, e.g. as determined a.m. Widmark at blood ethanol about 10 mmol/l (0.5 g/l). The reason for this could be increased ethanol elimination rates at the very high blood ethanol concentrations often reached in heavy drinkers.

We therefore examined the ethanol elimination rate in heavy drinkers following very high alcohol consumption (blood ethanol 40–70 mmol/l) and compared it to the elimination rate measured after intravenous administration of a smaller load of ethanol (blood ethanol 10 mmol/l).

Further, if a difference were found between these measurements of the elimination rates, we wanted to evaluate whether it could be ascribed either to concentration-dependence of a low-K_m pathway (such as the alcohol dehydrogenase system [1, 2] alone or in combination with a high-K_m pathway (such as the microsomal ethanol oxidizing system MEOS [3] or π-ADH [4]) or if increased activity of either of these systems could be involved—being a matter of current debate.

METHODS

Subjects

The material comprises alcoholic men, 'heavy drinkers' who came to the out-patient clinic for alcoholics after acute high alcohol intake. Eight subjects with blood ethanol concentrations above 40 mmol/l, with no history of liver disease, and who did not appear severely intoxicated, accepted to participate in the study during hospitalization. Informed consent to the procedures was obtained from each subject and the protocol was approved by the Ethical Committee of the Municipal Hospitals in Copenhagen.

All subjects claimed to have an average alcohol intake of no less than 100 g per day during at least one year and daily during the preceeding two weeks (see Table 1).

Case 2 had a regular daily intake of 30–60 mg chlordiazepoxide (Librium®) and Case 3 received phenobarbital 100 mg × 2 per day; none of the other subjects received drugs. Neither phenobarbital nor

Table 1. Data of alcoholic men, 'heavy drinkers'

Case no.	Age (yr)	Body weight (kg)	Alcohol intake		Chlordiazepoxide during 1st examination (mg)
			Years	Average g/day	
1	26	64	2–3	100	0
2	34	69	23	200–300	50
3	37	66	>15	200–300	0
4	28	85	3–4	400–500	25
5	34	72	16	300	50
6	59	73	15	>350	175
7	52	79	1	200–300	75
8	54	73	Several	300–600	75

other barbiturates were detectable in blood samples taken immediately after the arrival in any of the subjects.

All subjects appeared to be in a good state of nutrition and hydration, with no signs of liver diseases or other diseases at physical examinatiom. The heart rate during the first examination was on the average 85 beats/min (66–120) and the blood pressure 95/60–160/90. When sober the values were 70 beats/min (50–80), blood pressure 110/65–120/90.

The following liver tests were normal: prothrombine time [5], serum albumin concentration [5], galactose elimination capacity [6], antipyrine clearance [7] and serum bilirubin [5]. On admittance, serum aspartate aminotransferase [5] was elevated to four times the upper 95% limit of control values in two cases, and serum alkaline phosphatase [5] was elevated to two times the upper limit in one case; the following day all values were within control limits. Concentrations of glucose, Na^+, K^+ as well as pH, standard bicarbonate, arterial oxygen and carbon dioxide tensions in blood were normal.

Design of the study

First examination of the ethanol elimination. Blood sampling was started immediately after admittance to the hospital ward, i.e. 1–2 hr after stopping alcohol consumption, and between 10 a.m. and 4 p.m. Measurements from the first two hours were not included in the calculations. Venous blood samples were taken via a short indwelling Venflon® cannula from a peripheral arm vein every 20th minute for analysis of ethanol. The sampling was continued until blood ethanol, starting at 40–80 mmol/l, was below 2 mmol/l (12–20 hr). Approximately every 4th hour venous blood samples were taken for analysis of acetate, noradrenaline, and adrenaline.

The subjects had soft drinks, water and a few sandwiches during the measurement period. They were given oral doses of 25 mg chlordiazepoxide (Librium®) if necessary (see Table 1).

Second examination of the ethanol elimination. After a period of 1–3 days of abstinence, controlled during hospitalization, the ethanol elimination after a smaller dose of ethanol was studied (between 9 a.m. and 1 p.m.). In Cases 2 and 3 the intervals between the first and second examinations were for practical reasons 17 and 16 weeks, respectively, during which period both subjects were drinking alcohol as usual. Both subjects abstained from alcohol during hospitalization three days before the second examination. Case 1 declined to participate in the second examination.

Ethanol was given intravenously (about 400 mmol ethanol, 0.3 g/kg body weight, in 500 ml isotonic saline infused in the course of 12 min). Arterial blood samples were taken for analysis of ethanol from a peripheral vein every 7th minute for 63 min, starting 60 min after the beginning of the infusion with concentrations about 10 mmol/l. Venous blood samples for analysis of acetate, noradrenaline and adrenaline were taken at 21-min intervals.

Analytical procedures

Ethanol and acetate concentrations in blood were measured by enzymatic methods [8, 9].

Noradrenaline and adrenaline concentrations in plasma were measured by an isotope assay modified for small samples [10].

Calculations

The blood ethanol concentration–time curves (cf. Fig. 1) were evaluated in order to calculate the elimination rates of ethanol.

As a preliminary analysis we fitted linear regression lines for the concentration $c > 10$ mmol/l and for c between 2 and 10 mmol/l for the first examination, and between 2 and 10 mmol/l for the second examination in order to compare the slopes.

Next we analysed the data by a more detailed statistical analysis using a two pathway model

$$-\frac{dc}{dt} = \frac{V_{0.2} \cdot c}{0.2 + c} + \frac{V_{10} \cdot c}{10 + c}. \quad (1)$$

This describes two parallel Michaelis–Menten processes with $K_m = 0.2$ mmol/l and 10 mmol/l, respectively, symbolizing an ADH and a non-ADH pathway; the maximal elimination rates are denoted $V_{0.2}$ and V_{10}, respectively; c is concentration, dc/dt the slope of the concentration–time curve.

The differential equation (1) was solved to give c as a function of time t. This was performed by defining the function $g(x) = x + K_1 \ln(x) + K_2 \ln(x + K)$ with the auxillary parameters $K = (V_{0.2} \cdot K_{10} + V_{10} \cdot K_{0.2})/(V_{0.2} + V_{10})$, $K_1 = K_{0.2} \cdot K_{10}/K$ and $K_2 = K_{0.2} + K_{10} - K - K_1$. Integration of the equation (1) gives $c(t) = g^{-1}(g(c(0)) - (V_{0.2} + V_{10})t)$, see [11]. From

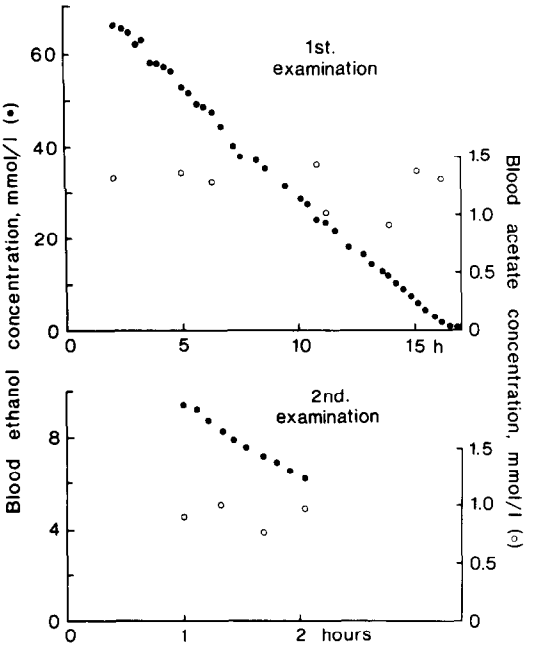

Fig. 1. Concentrations of ethanol and acetate in blood in Case 4 after intake of a large amount of alcohol (1st examination) and after i.v. infusion of a lower load of ethanol (2nd examination). Both the abscissa and the ordinate for the ethanol concentrations on the second examination are multiplied by a factor 5 so that direct comparison of the slopes can be made.

this we estimated the parameters $V_{0.2}$, V_{10}, and the concentration at time zero c (0) by fitting the curve to the data by the method of least squares. This gave values of the parameters as well as their relative errors (Table 2).

Next we wanted to evaluate if a one-pathway model with a low K_m value could describe the data adequately. We therefore fitted a one-pathway model with$K_m = 0.2$ mmol/l to the data (Table 3) and compared the values of the residual sum of squares (Q) with the Q values from the two-pathway model (Table 2) by F-tests.

RESULTS

Figure 1 gives an example of the blood ethanol concentrations from the first and the second examinations. The decrease of the concentrations at the first examination seems to be nearly constant from the high concentrations to about 10–5 mmol/l. Below this concentration there seems to be a slight bend of the curve. Comparison of the curves from both examinations seems to indicate a slower decrease during the second than during the first examination. The curves from the other subject show the same tendencies.

The elimination rates, determined in the preliminary analysis as the slopes of the curve, tended to be larger for $c > 10$ mmol/l than for c between 2 and 10 mmol/l at the first examination,but the difference was not statistically significant ($P > 0.05$). Comparison of the slopes for c between 2 and 10 mmol/l for the first and the second examinations showed a slight, but not statistically significant tendency for the elimination rate during the second examination to be smaller than during the first examination. The slope for $c > 10$ mmol/l at the first examination was significantly higher than the slope for c between 2 and 10 mmol/l at the second examination in 6 out of 7 subjects (each $P < 0.001$), indicating a higher elimination rate of ethanol at the high concentrations following massive alcohol intake than at the lower concentrations used at the routine procedure.

The two pathway model gave estimates of the overall maximal elimination rates $V_{0.2} + V_{10}$, determined with relatively small errors for the first examination (Table 2, third column). The large errors of the individual estimates of $V_{0.2}$ and V_{10} are due to difficulties of estimating their relative contribution to the total elimination rate. This is even more pronounced when the same procedure was applied to the second examination, not shown in the table.

The one-pathway model with K_m 0.2 mmol/l gave estimates of the maximal elimination rate with relatively small errors (Table 3). Comparison of the values of the residual sum of squares (Q) with those from the two-pathway model (Table 2) shows that the one-pathway model is as good as the two-pathway model for 6 of the 8 cases, the P-values being higher than 0.10 for 6 cases and around 0.05 for Cases 2 and 6 (the two-pathway model giving the best fit). Thus it is not possible to demonstrate a significant contribution of a high-K_m pathway in addition to a low-K_m pathway, but statistical uncertainty gives room for up to 50% elimination via a high-K_m pathway.

Finally we compared the elimination at the two examinations to see if there was any significant difference. Therefore we fitted, in view of the above results, a one-pathway model with identical values of $V_{0.2}$ for both the first and the second examination and compared the results with those of the above analysis with a one-pathway model with individual values of $V_{0.2}$. It was found that in six of the cases the model with individual values of $V_{0.2}$ for the two examinations gave significantly better fits to the data than the model with identical values of $V_{0.2}$; the P-values of the χ^2-tests were less than 0.001 in all cases except for Case 5 where it was 0.8. We therefore conclude that the elimination kinetics is significantly different following a high, respectively low load of ethanol. Under the assumption of a single low-K_m pathway, the large ethanol load results in a higher maximal elimination rate, on the average 49% higher (see Table 3).

The ethanol elimination rate following the low load of ethanol, being 58 μmol/min per l blood (S.E.M. = 3, $n = 8$) is of the same order of magnitude as the values in non-alcoholics examined by the same procedure, 56 μmol/min per l blood (S.E.M. = 9, $n = 19$ [12]).

The calculated total body elimination rate during the first examination was on the average 4.1 mmol/min, range 1.1–6.1 (calculated with the volume of distribution of ethanol of the second examination, see below). This is about the same as

Table 2. Ethanol elimination kinetics in alcoholic men with initial blood ethanol concentrations about 60 mmol/l (first examination) and about 10 mmol/l (second examination)*

Case no.	First examination $V_{0.2}$ (μmol/min per l blood) (relative error)	V_{10}	$V_{0.2} + V_{10}$	Q	df†	Second examination Q	df†
1	45 37%	45 62%	90 12%	58	26	—	—
2	25 64%	99 24%	124 6%	54	22	0.12	7
3	68 17%	17 86%	85 4%	52	37	0.25	7
4	59 11%	25 34%	85 3%	31	43	0.06	7
5	61 —	0 —	61 6%	26	27	0.33	7
6	39 23%	58 24%	97 5%	20	24	0.48	5
7	82 19%	21 123%	103 10%	12	21	0.65	7
8	104 —	0 —	104 9%	189	25	0.31	7
Mean			94				

* Maximal elimination rates are estimated by a model with two parallel Michaelis–Menten pathways, one with $K_m = 0.2$ mmol/l, and another with $K_m = 10$ mmol/l, denoted as $V_{0.2}$ and V_{10} respectively.

† The Q-values are the residual sum of squares with degrees of freedom (df) equal to the number of concentration measurements minus the number of parameters (= 3).

the claimed average daily intake (mean 104%, range 40–200%).

The average blood acetate concentration was in each case higher during the first examination (range 1.05–1.43 mmol/l, $n = 5$ subjects) than during the second examination (0.61–1.06 mmol/l, $P < 0.01$ (paired t-test), see Fig. 1 for an example).

Plasma noradrenaline concentrations were almost constant until blood ethanol was less than 2 mmol/l. During the first examination the mean was 0.39 ng/ml (S.E.M. = 0.07, mean of average values in four cases). This was significantly higher than during the second examination, 0.15 ng/ml (S.E.M. = 0.02, $n = 6$ cases, $P < 0.02$). The values from the second examination were similar to values in the normal fasting recumbent man (0.18 ng/ml, range 0.04–0.39, $n = 31$ [13]).

Plasma adrenaline, being on the average 36 pg/ml (S.E.M. = 15, $n = 4$) during the first examination, was not significantly different from values from the second examination, mean 31 pg/ml (S.E.M. = 10, $n = 6$). Both values are similar to control values (mean 40 pg/ml, range 0–20, $n = 31$ [13]).

DISCUSSION

The main finding of the study is that the ethanol elimination rate is higher at the high concentration after acute massive alcohol intake in the present 'heavy drinkers' than the elimination rate measured in the same subjects by the routine procedure with intravenous ethanol administration to a lower concentration after a few days of abstinence during hospitalization. The calculation of the elimination rates is based on a certain number of assumptions (see below); by assuming elimination via one pathway (with $K_m = 0.2$ mmol/l) or just by comparing the slopes of the ethanol concentration–time curves, we find that the elimination rate is significantly higher (about 49%) following the heavy drinking than at the lower concentrations used routinely. Furthermore, the calculated elimination rate at the high concentration corresponds roughly to the claimed daily intake. And the elimination rate at the lower concentration corresponds to the values measured in non-alcoholics using the same procedure.

In the statistical analysis of the decrease in the

Table 3. Ethanol elimination kinetics estimated by a one-pathway model with $K_m = 0.2$ mmol/l and maximal elimination rate $V_{0.2}$

Case no.	First examination $V_{0.2}$*	$Q_{0.2}$ $df_{0.2}$†	$V_{0.2}$*	$Q_{0.2}$ $df_{0.2}$†
1	72 3%	64 27	—	
2	95 3%	66 23	71 6%	0.37 8
3	81 1%	54 38	42 7%	0.25 8
4	78 1%	39 44	53 3%	0.09 8
5	61 1%	26 28	61 6%	0.39 8
6	78 2%	34 25	55 12%	0.48 6
7	95 2%	12 22	61 8%	0.65 8
8	104 3%	189 26	60 7%	0.45 8
Mean	83		58	

* Maximal elimination rate: μmol/min per l blood (relative error).

† $Q_{0.2}$ is the residual sum of squares and $df_{0.2}$ the degrees of freedom, see Table 2.

blood concentration at the first examination it is not necessary to involve a second high-K_m pathway. On the other hand, the analysis of the data cannot exclude that up to 50% of the elimination does take place via such a pathway. The finding of different elimination rates at both examinations cannot be explained solely by concentration-dependent elimination according to Michaelis–Menten saturation kinetics with either a low or a high K_m. The findings are compatible with a higher activity (V_{max}) of a low K_m pathway during the first examination than during the second examination, the elimination remaining high throughout the decrease of the ethanol concentration from 40–80 mmol/l to about 2 mmol/l.

In evaluating the blood concentration curves from both examinations and by comparing them, a number of assumptions have to be made. The values used for K_m are based on determinations *in vitro* [1, 3, 4] and *in vivo*[2]. No attempt was made to estimate K_m in the present study, since this requires measurements at very low blood concentrations and measurements of hepatic blood flow [2, 16]. The parameters V_{max} and K_m used in the present context are applied to the kinetics of the complex situation in the intact organism and are not necessarily directly comparable with the parameters estimated *in vitro* with other conditions for the enzymes.

The kinetic models used—with one or two Michaelis–Menten pathways—are of course simplifications of the complex physiological and metabolic conditions in the intact organism. These simplifications are, however, necessary to be able to estimate parameters with evaluation of the statistical accuracy. By using the kinetic models we become furthermore able to utilize data from both high and low concentrations in the same analysis—in contrast to the use of the simple linear regressions.

The use of values of elimination rates in terms of μmol/·min per·l blood presumes that the volume of distribution was the same for each subject at the first and the second examination. This cannot be evaluated from the present data.

We further assume that the ethanol blood concentration measured 2 hr after admittance, i.e. 3–4 hr after stopping drinking, is not influenced by continued intestinal absorption of alcohol and distribution phenomena. It is also assumed that initial distribution phenomena have diminished so much that the procedure of taking venous blood samples at the first examination and arterial samples at the second examination do not bias the comparison of the curves [14]. It may be remarked that for ethical reasons we were not able to give high intravenous doses of ethanol like those consumed by the subjects.

The estimated ethanol elimination rates include not only hepatic metabolism but also elimination in expired air, urinary excretion and other sources of extrahepatic elimination. We evaluated the respiratory loss in the analysis of the two-pathway model by including a term of first-order elimination based on the air: blood partition coefficient of ethanol 1:2300 [15], an alveolar ventilation of 4.3 l/min and the volume of distribution of ethanol (L, litre blood) by the equation

$$-L\cdot\frac{dc}{dt}=\frac{V_{0.2}\cdot c}{0.2+c}+\frac{V_{10}\cdot c}{10+c}+f\cdot c \qquad (2)$$

where $f = 4.3/2300$ l/min and $V_{0.2}$ and V_{10} (mmol/min) are the respective maximal elimination rates with K_m values 0.2 and 10 mmol/l. We calculated L = the infused amount/$c(0)$ from the second examination. Just as for the model equation (1) we can express the solution as a function of c on t [11] and estimate the parameters $V_{0.2}$, V_{10}, $c(0)$ by the method of least squares.

The analysis showed that the amount of ethanol expired constituted less than 5% of elimination; this is not significant in the present context. It may be noticed that the calculated volume of distribution from the second examination was on average 60% of the body weight, corresponding to what is usually found [12].

The extrahepatic elimination of ethanol has been judged to be up to one third of the total elimination at 2–10 mmol/l in subjects with cirrhosis [16], but its kinetics is unknown. The urinary concentration of ethanol is about the same as in plasma [17], and thus urinary excretion can maximally account for 1–3% of the total elimination depending on the diuresis.

It should be noticed that possible changes in the hepatic blood flow rate during the high ethanol concentrations and during increased noradrenaline concentrations will not influence the present result since the maximal elimination rate is independent of the hepatic blood flow rate [18].

The higher acetate concentrations after the heavy ethanol load than after the lower load support the observation of a higher ethanol elimination rate after the heavy load since the peripheral acetate elimination equals the hepatic production of acetate which again equals the hepatic ethanol elimination [19]. This argument requires steady-state which was approximately achieved (see Fig. 1).

The elevated plasma noradrenaline concentrations during the first examination indicate that the sympathetic nervous activity was stimulated during the high ethanol concentrations whereas adrenaline secretion remained unchanged. The normal concentrations of noradrenaline during the second examination indicate that high ethanol concentrations are required for the stimulation.

It may be hypothesized that very high ethanol concentrations stimulate the hepatic ethanol metabolism via a stimulation of the sympathetic nerve system, concomitantly enhanced hepatic oxygen uptake [20]—and increased capacity for mitochondrial reoxidation processes [21]. Furthermore it could be speculated that the stimulatory effect takes place via a low-K_m system, probably ADH. In agreement with this, Wendell and Thurman [22] found that the rate-accelerating effect of high ethanol concentrations was nearly totally abolished by the ADH-inhibitor pyrazole.

We have tried to stimulate the ethanol elimination by simultaneous infusions of ethanol and noradrenaline into two healthy volunteers (M. Parm and S. Keiding, unpublished data). There was, however, no change of the ethanol elimination rates in spite of increments of plasma noradrenaline from about 0.2 ng/ml up to 1.4 ng/ml and 2.9 ng/ml, respectively. These studies thus cannot give direct support to the above hypothesis.

The period of alcohol abstinence of 1–3 days between the first and the second examination may be of importance for the observed lower elimination rate during the second examination, whether being related to the increased noradrenaline concentration during the first examination or to a decrease in a possible enhanced enzyme activity during the period of heavy drinking. In this connection it is interesting to note that Lindros (personal communication) has observed a decrease in ethanol elimination rate in alcoholics after 1 week of abstinence.

The question whether the stimulatory effect of the high ethanol concentrations depends on a daily high ethanol intake causing induction phenomena [3] or if it is present also in non-alcoholics [23] cannot be answered from the present data. It is, however, interesting to note that Korsten *et al.*[24] have found nearly identical elimination rates in alcoholics and non-alcoholics after intravenous ethanol administration to blood concentrations about 50 mmol/l, being on the average 84 μmol/min and 89 μmol/min, respectively, and being of the same order as our values at the high concentrations (see Table 2). This is in agreement with the present hypothesis that the very high ethanol concentrations stimulate the ethanol metabolism—perhaps via ADH, and perhaps related to the demonstrated increased activity of the sympathetic nerve system.

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