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Curvilinear increase in methanol concentration after inhibition of oxidation by ethanol: significance for the investigation of endogenous methanol concentration and formation

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Abstract Endogenous methanol production was assessed over a period of 5 h in subjects given an infusion of ethanol to inhibit methanol oxidation in the liver after a period of fasting and abstinence from alcohol. Ethanol was administered to each of five subjects at rates of 0.35 g/kg per hour and 0.70 g/kg per hour. The rise in methanol concentration was biphasic regardless of the rate of ethanol administration, with a steeper gradient in the first 10–30 min. This may be due to the existence of a deep compartment from which methanol can be displaced by ethanol. This could take the form of loose binding of methanol to the hepatic oxidation enzymes as an enzyme-substrate complex, or a shift of the oxidation-reduction equilibrium between methanol and formaldehyde. The biphasic nature of the increase, with an initial steeper rise, means that the values obtained in the first 30 min should be excluded from the calculations when the rate of endogenous methanol production is determined by linear regression analysis. Endogenous methanol concentrations to be taken into account after ethanol administration are on average 0.4–0.6 mg/kg higher than those detectable in the absence of ethanol due to the additional method displaced from the deep compartment.

Key words Endogenous methanol · Concentration · Production · Deep compartment

Introduction

Blood methanol levels are of forensic interest because of their significance in the areas of congener alcohol analysis and diagnosis of alcoholism. Most of the methanol in the body is derived from the consumption of alcoholic beverages, but there is also some endogenous production. The latter can be demonstrated experimentally by blocking methanol oxidation in the liver by the administration of ethanol. The subsequent rise in methanol concentration has generally been described as linear and the rate of synthesis as constant (Iffland et al. 1985, 1989; Gilg et al. 1987; Jones et al. 1987; Bilzer et al. 1990; Gilg 1992). We have recently published results on the variability in the rate of endogenous methanol production, suggesting that the rise in methanol concentration when oxidation is blocked by ethanol may not be strictly linear (Haffner et al. 1996). This would be of consequence in the investigation of endogenous methanol levels and production.

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Material and methods

The study was performed on six healthy male subjects between 20 and 24 years old, who were accustomed to drinking alcohol. The investigations were approved by the Ethics Committee of the Faculty of Medicine, University of Essen. The investigations were carried out on two separate occasions using two different rates of ethanol administration. Of the subjects four took part on both days, and two participated on only one day, so that a total of five sets of results were obtained with each method.

The subjects ate lunch on the day before the investigation and were denied solid food thereafter. In the evening, they were given 20–30 g of Glauber's salt with ample fluid to clear the bowel and circumvent or at least minimize the effects of dietary pectin (Grüner and Bilzer 1983; Gilg 1992; Grüner et al. 1994) and metabolic processes in the intestinal flora (Stöhlmacher 1995).

The investigation commenced at about 1.00 pm on the next day. Methanol oxidation was blocked by the administration of a methanol-free 7g% isotonic ethanol solution through a volumetric infusion pump at rates of 0.35 g/kg body weight per hour and 0.70 g/kg body weight per hour. The blood ethanol concentration was monitored indirectly by measurement of ethanol concentrations in expired air. A blood ethanol concentration of about 0.5 g/kg was reached after about 80 min (or 40 min for the faster infusion rate). The rate of infusion was then reduced to 0.1 g/kg body weight per hour to maintain the blood ethanol concentration at this level for the remaining 5 h of the investigation.

Blood samples were taken from an intravenous cannula in the opposite arm to the ethanol infusion for determination of the serum

methanol and blood ethanol concentrations immediately before ethanol infusion was commenced at 5, 10, and 20 min after commencement and at 20-min intervals thereafter. The samples were centrifuged and the serum pipetted off and stored at about 4° C until processed. Ethanol and methanol concentrations were determined by gas chromatography (ethanol: 0.4% Carbowax 1500 on graphite 60/80 mesh 2 m 1/8″; methanol: 5.5% Carbowax 20 M on Carbopack B/Supelco 1–1766), a mean value being derived from two readings for each parameter. Tertiary butanol was used as an internal standard. Quantitative evaluation of the methanol concentration was performed by the addition method in which a defined amount of methanol is added. This method has a within-run precision of ± 10% at 1 mg/kg. Medidrug BGS S (2.5 mg/kg) was used as an external standard. Although methanol concentrations in the serum are generally quoted in the literature, ethanol concentrations usually refer to whole blood. Therefore, for comparability measured serum ethanol concentrations were converted to blood concentrations by dividing the GC integration values by a factor of 1.256 (Machata 1967).

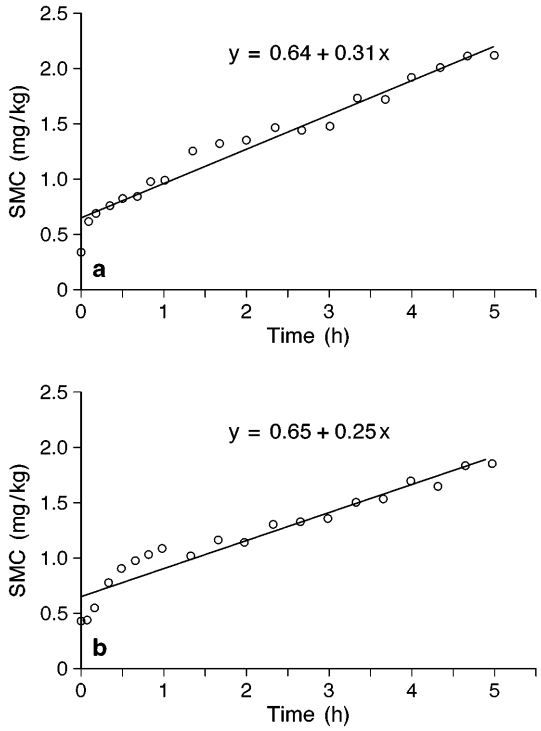

Fig. 1 Changes in serum methanol concentration (SMC) in the presence of ethanol: mean observed values and linear regression line (y = a + bx) derived from these values. Initial rate of ethanol infusion: **a** 0.35 g/kg body weight/h, **b** 0.70 g/kg body weight/h

Results

The mean endogenous methanol concentration (i.e. at t = 0, before ethanol infusion) was 0.37 ± 0.126 mg/kg (median, 0.35 mg/kg; range 0.24–0.57 mg/kg). After ethanol infusion was commenced, the methanol concentration increased. The rise appeared to be slightly steeper during the initial period (Fig. 1).

We first assumed the rise in concentration to be linear as is normal practice and established linear regression equations (y = a + bx) for the whole period of observation (t = 0–300 min; Fig. 1; Table 1). However, it subsequently became apparent that the endogenous methanol concentration at t = 0 is not represented by the regression equation. All the readings for this value lay below the intercept of the regression line and, with one exception, outside the 95% confidence interval.

We then checked the linearity of the curves of all the subjects using the method of residual values. The residual, i.e. the difference between the measured value and the theoretical value derived from the equation, was determined for each value in each subject and plotted against time, two different plots being derived for the two different initial rates of infusion (Fig. 2). If a mathematical model subjected to this test represents the measured values accurately, the residuals will be found to be distributed randomly about the X-axis along its whole length. It is obvious from Fig. 2 that this was not the case for the initial part of the experiment. As can be seen more clearly with the faster rate of infusion, the residuals for the first few measurements were all negative, and there was a compensatory shift to the positive side in those directly thereafter. Random distribution did not occur until later. The rise in methanol concentration cannot therefore be considered linear over the whole of the observation period. It appears that an additional process is superimposed during the initial steeper rise in methanol concentration. We therefore derived nonlinear regression equations (y = c + dx/(e + x) + fx) from the readings. Here, c represents

Table 1 Endogenous methanol concentration (SMC) and production after inhibition of oxidation by ethanol: linear regression equations (y = a (mg/kg) + b (mg/kg/h) × (h)) for the period t = 0–300 minutes (n = 20)

Subject	Initial rate of ethanol infusion	Observed SMC at t = 0	Linear regression equation for t = 0 – 300	95% confidence interval	
				a	b
1	0.35 g/kg/h	0.26 mg/kg	y = 0.39 + 0.28x	± 0.05	± 0.02
1	0.70 g/kg/h	0.30 mg/kg	y = 0.49 + 0.32x	± 0.09	± 0.03
2	0.35 g/kg/h	0.25 mg/kg	y = 0.44 + 0.36x	± 0.11	± 0.04
2	0.70 g/kg/h	0.25 mg/kg	y = 0.49 + 0.28x	± 0.10	± 0.04
4	0.35 g/kg/h	0.40 mg/kg	y = 1.38 + 0.40x	± 0.26	± 0.10
4	0.70 g/kg/h	0.51 mg/kg	y = 0.70 + 0.30x	± 0.11	± 0.04
6	0.35 g/kg/h	0.50 mg/kg	y = 0.56 + 0.18x	± 0.10	± 0.04
6	0.70 g/kg/h	0.42 mg/kg	y = 0.62 + 0.19x	± 0.15	± 0.06
5	0.35 g/kg/h	0.24 mg/kg	y = 0.44 + 0.35x	± 0.10	± 0.04
3	0.70 g/kg/h	0.57 mg/kg	y = 0.96 + 0.15x	± 0.18	± 0.07

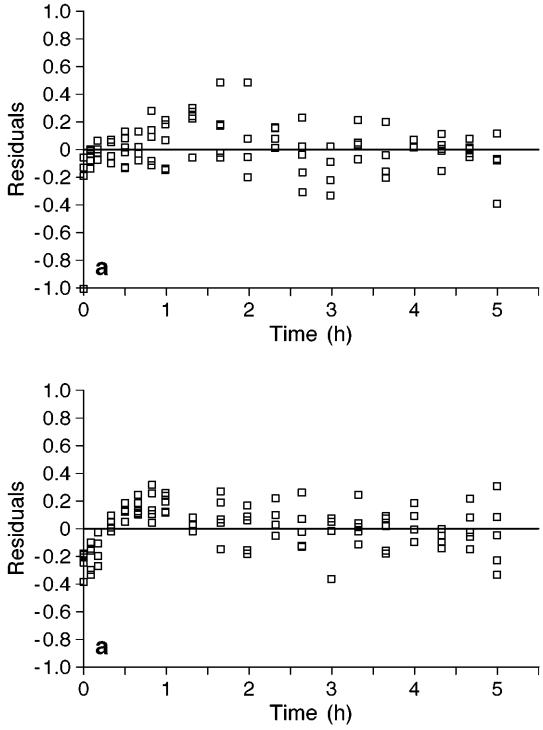

Fig. 2 Deviation (residual values) of linear regression line for t = 0–300 min. Initial rate of ethanol infusion: **a** 0.35 g/kg body weight/h, **b** 0.70 g/kg body weight/h

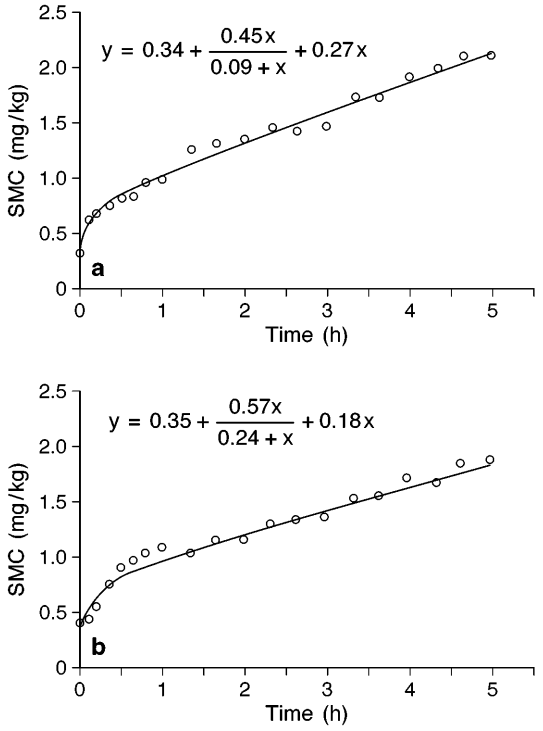

Fig. 3 Changes in serum methanol concentration (SMC) in the presence of ethanol: mean observed values and nonlinear regression line (y = c + dx/(e + x) + fx) derived from these values. Initial rate of ethanol infusion: **a** 0.35 g/kg body weight/h, **b** 0.70 g/kg body weight/h

the endogenous methanol concentration before enzyme inhibition, and fx the later, linear increase in methanol concentration. The hyperbola dx/(e + x) characterizes the additional superimposed process occurring at the beginning, d representing the magnitude of the additional increase and e the half-life of this additional increase (Fig. 3).

The formula associated with an initial rate of ethanol infusion of 0.35 g/kg per hour was: y = 0.34 + 0.45x / (0.09 + x) + 0.27x, and that for 0.70 g/kg per hour was y = 0.35 + 0.57x / (0.24 + x) + 0.18x, corresponding to endogenous methanol levels of 0.34 mg/kg and 0.35 mg/kg, respectively, and a later linear increase in methanol concentration of 0.27 mg/kg per hour and 0.18 mg/kg per hour, respectively. The superimposed process leads to a further increase of about 0.45 mg/kg and 0.57 mg/kg, respectively, which occurs during a period of about 10–30 min.

These findings indicate that the rise in endogenous methanol levels after oxidation inhibition can be considered a biphasic process. A biochemical or biophysical process occurs during the first 10–30 min of ethanol infusion that is superimposed on the pre-existing endogenous methanol concentration and on the endogenous methanol production, and results in an initial steeper rise in concentration (Fig. 4). Not until after this phase do the changes in concentration appear to be dependent only on the rate of

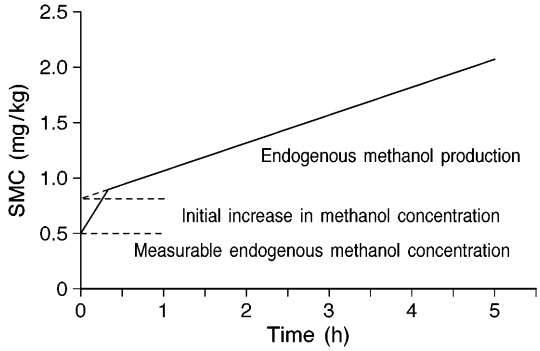

Fig. 4 Biphasic increase in methanol concentration after blockade of oxidation by ethanol (theoretical model)

Table 2 Endogenous methanol production after inhibition of oxidation by ethanol: linear regression equations (y = a (mg/kg) + b (mg/kg/h) × (h)) for the period t = 30 – 300 minutes (n = 16)

Subject	Initial rate of ethanol infusion	Linear regression equation for t = 30 – 300	95% confidence interval	
			a	b
1	0.35 g/kg/h	y = 0.42 + 0.27x	± 0.06	± 0.02
1	0.70 g/kg/h	y = 0.60 + 0.29x	± 0.10	± 0.03
2	0.35 g/kg/h	y = 0.50 + 0.35x	± 0.17	± 0.06
2	0.70 g/kg/h	y = 0.65 + 0.23x	± 0.07	± 0.02
4	0.35 g/kg/h	y = 1.65 + 0.32x	± 0.26	± 0.09
4	0.70 g/kg/h	y = 0.83 + 0.26x	± 0.11	± 0.04
6	0.35 g/kg/h	y = 0.61 + 0.17x	± 0.16	± 0.05
6	0.70 g/kg/h	y = 0.70 + 0.16x	± 0.22	± 0.07
5	0.35 g/kg/h	y = 0.48 + 0.33x	± 0.15	± 0.05
3	0.70 g/kg/h	y = 1.22 + 0.08x	± 0.15	± 0.05

endogenous production, which is represented most simply by the linear regression equation calculated from the measurements for the period t = 30–300 min (Table 2).

Discussion

Endogenous methanol levels represent the balanced result of endogenous production and elimination, the latter occurring through oxidation by alcohol dehydrogenase (ADH) and the microsomal ethanol-oxidizing system (MEOS), especially cytochrome P450–II–E1, and excretion through the lungs and kidneys. If oxidation is blocked by ethanol, the methanol concentration rises until a new steady state between endogenous production and excretion is reached. This new equilibrium occurs at a serum concentration of about 10 mg/kg (Gilg 1992).

Experiments on chimpanzees have shown that the new steady state is reached in about 4–5 days (Pieper and Skeen 1972) and the rise takes the form of a saturation curve. Investigations involving human subjects, however, are usually continued for only a few hours and it is customary, and theoretically justified, to represent the increase during this short period as a linear function – despite the fact that the gradient over the total period in fact falls to zero asymptotically – the intercept "a" in the formula $y = a + bx$ representing the endogenous methanol level, and the gradient "b" the rate of synthesis.

Our findings, however, indicate that the applicability of this model is limited. A linear rise in methanol levels due solely to endogenous production, is not seen until 10–30 min after the start of the oxidation inhibition. Before this point, the methanol concentration rises more rapidly than can be accounted for by endogenous production alone and it appears that an additional biochemical or biochemical/biophysical process also occurs during this period.

This postulated process leads to an increase in the methanol concentration of about 0.4–0.6 mg/kg. The duration is short and this length of time could be needed mainly for distribution, because according to our own findings (unpublished) the distribution half-life for methanol is about 10 min. True biochemical synthesis therefore appears an unlikely explanation. It is more probable that there is a deep compartment representing a reservoir in which methanol exists in an undetectable form, from which it is expelled by ethanol. It is possible, for example, that this could take the form of loose binding of methanol, at a concentration of 0.4–0.6 mg/kg (corresponding to about 0.012–0.018 mMol/kg) to the alcohol oxidizing enzymes of the liver. The flood of ethanol would quickly displace methanol from this enzyme-substrate complex, because ethanol not only has a greater affinity for the enzyme (von Wartburg et al. 1965; Blair and Vallee 1966; Mani et al. 1970) but is also infused at a rate that produces a concentration 100 times that of methanol. It is also possible that there is a shift in the oxidation-reduction equilibrium between methanol and formaldehyde as a result of the flood of ethanol. The endogenous formaldehyde concentration is about 2.6 mg/kg, or about 0.1 mMol/kg (Heck et al. 1985). The balance lies in favour of formaldehyde, and even a slight shift in the other direction would be enough to account for the rise in methanol concentration.

The pathway of endogenous methanol production has not yet been identified with certainty. Various theories have been developed (Axelrod and Daly 1965; Gilg et al. 1987; Stöhlmacher 1995) and it is conceivable that several different pathways are involved. It is therefore theoretically possible that the steeper rise in concentration during the early phase of ethanol infusion can also be explained by the inhibition of one of several synthetic pathways by ethanol. However, even in the absence of ethanol, greater methanol levels would be expected in expired air and urine.

If, as our findings suggest, the rise in methanol concentration after oxidation inhibition by ethanol is biphasic, with a more rapid rise at the outset, this would have consequences for investigations on the rate of endogenous methanol production and for the endogenous methanol concentration to be taken into account in congener alcohol analysis. If the rate of methanol production is calculated from the gradient of a linear regression line for the whole period of observation, the systematically lower values in the initial phase would tend to increase the value obtained. This purely mathematical error becomes more accentuated, the greater the proportion of the measured values lying within the first 30 min. However, the usual experimental design in this field of study, with half-hourly or hourly blood sampling over observation periods of several hours, would probably make this error negligible, since only the first value (t = 0) would be discrepant. This is also borne out by the fact that the more accurate method for calculation of the rate of methanol synthesis used in this study did not produce values substantially different from those derived in other studies (Iffland et al. 1985, 1989; Gilg et al. 1987; Jones et al. 1987; Bilzer et al. 1990; Gilg 1992).

A greater effect can be expected in the evaluation of endogenous methanol levels. These have generally been investigated in subjects who have undergone a period of ethanol abstinence, mean serum concentrations of 0.9 mg/kg being found under normal nutritional conditions (Iffland et al. 1985, 1989; Gilg 1992; Grüner et al. 1994) and 0.3–0.4 mg/kg after a longer period of fasting (Haffner et al. 1996), as in this study. However, the findings of this study indicate that this only includes the "free" endogenous methanol fraction. The fraction concealed in the postulated deep compartment appears first in the initial phase of ethanol administration. The total endogenous methanol concentration that then appears can best be calculated mathematically from the intercept of the linear regression equation derived from the period t = 30–300 min, or alternatively by nonlinear regression, as described above. It was found in this study to be 0.4–0.6 mg/kg higher than the conventionally dervied value for "free" methanol concentration measured under conditions of abstinence from ethanol.

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