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Hepatic ethanol elimination kinetics in patients with cirrhosis

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Abstract

Objective—To address the question of whether increased ethanol elimination in alcoholics can be ascribed to increased metabolism via alcohol dehydrogenase (ADH; K_m around 0.2 mM) or the microsomal ethanol-oxidizing system (MEOS; K_m 10 mM) by kinetic analysis of hepatic ethanol elimination in recently drinking patients with alcoholic cirrhosis and healthy subjects. A further objective was to investigate whether systemic clearance of ethanol at low arterial ethanol concentrations can be used as a measure of hepatic blood flow.

Material and methods—Six patients with alcoholic cirrhosis were enrolled after 2 days of abstinence, along with 6 healthy subjects. Ethanol was administered as 6 successive infusions in increasing doses. Arterial and hepatic venous blood concentrations of ethanol were measured; hepatic blood flow was measured simultaneously. Kinetic parameters were calculated according to the sinusoidal perfusion model of enzymatic elimination by the intact liver.

Results—Mean hepatic K_m for ethanol was 0.16 mM (range 0.09–0.36) in healthy subjects and 0.36 mM (range 0.16–0.69) in patients with cirrhosis ($p>0.3$), both compatible with the K_m for ADH. The two groups of subjects had similar V_{max} values ($p>0.3$). Extrahepatic elimination of ethanol accounted for more than 50% of total elimination in both groups, which precludes the use of systemic clearance as a measure of hepatic blood flow.

Conclusions—The results support the hypothesis that ADH remains the main pathway for hepatic elimination of ethanol in recently drinking patients with alcoholic cirrhosis. We interpret this as evidence against a significant contribution of MEOS *in vivo*.

Keywords

Alcoholism; alcohol dehydrogenase; liver metabolism; liver pathophysiology; microsomal ethanol oxidizing system

Introduction

In the liver, ethanol is converted to acetaldehyde by the cytosolic alcohol dehydrogenase enzyme [1,2] (ADH; K_m around 0.2 mM [2–4]) or the microsomal ethanol-oxidizing system [5–7] (MEOS; K_m around 10 mM [5–9]). Increased MEOS activity found in liver tissue and increased ethanol clearance *in vivo* in rats fed an ethanol-rich diet [5,8] led Lieber & DeCarli to suggest that increased elimination of ethanol in human alcoholics can be ascribed to

increased MEOS activity. However, in subsequent studies in rats [10,11], the investigators found no significant contribution of MEOS *in vivo* compared to alcohol dehydrogenase (ADH). Similarly, in heavy drinking subjects with very high blood concentrations of alcohol [12], we found that the blood ethanol concentration disappearance curve was compatible with a total body ethanol elimination via ADH [2-4] with no significant contribution of MEOS [5,6,8,9]. Unfortunately, our paper [12] has been misquoted for indicating an important contribution of MEOS in humans *in vivo* [13-15]. Various reviews also describe increased elimination of ethanol in alcoholics as being due to increased MEOS activity [13,16-18] although, as far as we know, this has never been demonstrated experimentally in humans. We therefore sought to determine K_m of the hepatic ethanol metabolism in alcoholic human subjects and healthy subjects to test the hypothesis that hepatic ethanol metabolism is compatible with ADH as the main pathway in both groups of subjects. We applied the classical physiological methodology with successive constant intravenous infusions of ethanol in increasing doses and measurements of approximate steady-state blood concentrations of ethanol in arterial and hepatic venous blood. Kinetic parameters of the hepatic ethanol elimination (i.e. splanchnic ethanol elimination) were calculated according to the sinusoidal perfusion model, which describes *in vivo* enzymatic elimination of substrates by the intact liver [19-22].

In order also to address the question of whether ethanol clearance can be used as a measure of hepatic blood flow [23,24], we designed the study to obtain good approximation to low steady-state blood ethanol concentrations in the first low-concentration measurement period.

Material and methods

Subjects

Five hospitalized patients with alcohol-induced, biopsy-proven cirrhosis were enrolled after 2–3 days of abstinence. Patients with viral or autoimmune cirrhosis were not included. In the liver patients, liver vein catheterization was performed for measurements of the hepatic venous pressure gradient. Four healthy subjects with no history of alcohol abuse were recruited through an advertisement in a local newspaper. The metabolic liver function was assessed by means of the galactose elimination capacity (GEC) [25,26]. In the data analysis, we included results from a previous study from our group [19], comprising 1 patient with alcoholic liver cirrhosis and 2 subjects with no liver disease, who were examined according to the same protocol as that used in the present study.

Informed written consent was obtained from each subject and the study protocol was approved by the local ethics committee. The studies were conducted in compliance with the Declaration of Helsinki II ethics guidelines. No complications of the procedures were observed.

Catheterization and measurement of liver blood flow

The subjects fasted for 8 h. A Courmand catheter (Surgimed, Copenhagen, Denmark) was placed in a right liver vein through an introducer catheter placed percutaneously in a cubital vein. The position of the tip of the Courmand catheter was verified by fluoroscopy using 5 ml contrast (Omnipaque, 240 mg ml⁻¹, Nycomed DAK, Roskilde, Denmark) [27]. An Artflon catheter (Becton Dickinson, Swindon, UK) was placed percutaneously in a radial artery.

Indocyanine green (ICG; Hynson, Westcott and Dunning, Baltimore, USA) was given as a constant intravenous infusion (0.20 $\mu\text{mol min}^{-1}$) throughout the experiment, starting 90 min before the first measurement period [28]. Arterial and liver vein blood samples for analysis of plasma concentrations of ICG [29,30] were drawn simultaneously with the samples for the measurements of the blood concentration of ethanol. Hepatic blood flow, F (l blood

min^{-1}) was calculated for each of the measurement periods according to the Ficks principle [28] as the ICG infusion divided by the difference between mean arterial and liver vein plasma concentrations of ICG, and corrected for haematocrit. Individual mean values of hepatic blood flow ranged from 1.12 to 2.85 l min^{-1} (median, 1.29 l min^{-1}) in the patients with cirrhosis and from 1.03 to 1.56 l min^{-1} (median, 1.31 l min^{-1}) in the healthy subjects; the difference was not significant ($p>0.3$). The individual flow values did not vary significantly during the time-course of the experiment.

Ethanol kinetics

Each study comprised 5–7 measurement periods with successive constant intravenous infusions of ethanol in increasing doses ranging from 0.18 to 1.90 mMol min^{-1} (Figure 1).

The initial low-dose period was preceded by an equilibration infusion of 90 min in order to ensure good approximation to steady-state so that it could be determined whether ethanol clearance can be used as a measure of hepatic blood flow. Each of the subsequent measurement periods lasted 16–30 min and was preceded by an equilibration period of 25–35 min. Blood samples were taken simultaneously from the artery and the liver vein every 3rd to 6th minute during each of the measurement periods for analysis of blood concentrations of ethanol [31] and ICG [29,30].

Mean values of the blood concentrations of ethanol in the artery (A; mM) and the liver vein (V; mM) in each of the 5–7 measurement periods were used to calculate the hepatic elimination rate of ethanol, $v=F(A-V)$ (mmol min^{-1}), and the logarithmic average sinusoidal blood concentration of ethanol, $\hat{c}=(A-V)/\ln(A/V)$ (mM) [19–22]. The hepatic maximal elimination rate of ethanol, $V_{\max}$ (mmol min^{-1}) and the Michaelis constant K_m (mM), were calculated according to the sinusoidal perfusion model of enzymatic elimination of substrates by the intact liver [19–22] by non-linear regression of the model ($v=V_{\max} \hat{c}/(K_m+\hat{c})$) to the individual data sets of v and $\hat{c}$.

Mean values of K_m and $V_{\max}$ from the two groups of subjects were compared using Student's *t*-test, and a *p*-value of less than 0.05 was considered as an indication of statistically significant difference.

From the first measurement period, the hepatic extraction fraction of ethanol was calculated as $(A-V)/A$ and the extrahepatic elimination of ethanol was calculated by subtracting the hepatic elimination rate of ethanol from the infusion rate.

Results

The patients had significantly reduced metabolic liver function as assessed by GEC, being on average two-thirds of the values in the healthy subjects (Table I).

Hepatic K_m for ethanol ranged from 0.16 to 0.69 mM in patients with alcoholic cirrhosis and from 0.09 to 0.36 mM in healthy subjects (Table I); the difference was not significant ($p>0.3$). Hepatic $V_{\max}$ for ethanol elimination ranged from 1.04 to 1.95 mmol min^{-1} in patients with alcoholic cirrhosis and from 1.12 to 2.09 mmol min^{-1} in healthy subjects (Table I); again, the difference was not significant ($p>0.3$).

The arterial blood concentration of ethanol in the first measurement period was 0.05–0.15 mM (median, 0.06 mM) and varied by less than 1%/h in each subject, indicating good approximation to steady-state (Figure 1). The extrahepatic elimination of ethanol in this period encompassed 44–66% (mean 54%) of the total elimination of ethanol in the patients with cirrhosis and 46–63% (mean 55%) in the healthy subjects; the difference was not

significant ($p>0.3$). The hepatic elimination in both groups hence accounted for less than 50% of the total elimination at low ethanol concentrations.

Discussion

The results support our working hypothesis that the hepatic elimination of ethanol is governed primarily by an enzymatic pathway with a low K_m in both patients with alcoholic cirrhosis and healthy subjects. The *in vivo* K_m values, being on average 0.36 mM in the cirrhotic patients and 0.16 mM in the healthy subjects (Table I), are comparable with *in vitro* estimates for human liver ADH of around 0.2 mM [2-4] and previous *in vivo* estimates of around 0.2 mM in humans with cirrhosis [12] and in humans without liver disease [12,32]. This observed K_m is significantly lower than the *in vitro* K_m of 7–20 mM for human MEOS [5-9]. The study thus indicates that the hepatic elimination of ethanol in recently drinking patients with cirrhosis can be described adequately by metabolic conversion by the ADH enzyme alone, in agreement with previous findings in humans [12] and rats [10,11,33].

The average K_m in the patients with cirrhosis was 2–3 times higher than that in the healthy subjects, although the difference was not statistically significant. It is noteworthy, however, that structural heterogeneity with intrahepatic vascular shunting and distorted vascular structure of the cirrhotic liver [27,34] will give rise to less efficient elimination and consequently too high estimates of the K_m as described in the distributed sinusoidal perfusion model of enzymatic elimination [35]. In other words, K_m in the hepatocytes in the diseased livers might actually be even lower than the calculated values, i.e. more similar K_m for AHD.

The finding of similar values of V_{max} of the hepatic elimination of ethanol in patients with cirrhosis and in healthy subjects is in agreement with findings by Winkler et al. [36] but is in contrast to the significantly reduced estimates of liver V_{max} of galactose (GEC) in the cirrhotic patients (Table I). This apparent paradox can be interpreted as an example of dissociated impairment of liver metabolic functions during liver cell impairment. The normal V_{max} for ethanol, despite a reduced liver function (as assessed by the reduced GEC), can be ascribed to induction of ADH by alcohol, being in agreement with the findings in alcohol-fed rats [33] and drinking humans [12], whereas the impaired GEC is a result of loss of functional liver mass.

The extrahepatic ethanol elimination of about 55% of total elimination in the first measurement period with low arterial blood concentrations of ethanol (median 0.06 mM; range 0.05–0.15 mM) is somewhat higher than that observed in patients with cirrhosis at blood ethanol concentrations of about 5 mM, estimated to be up to one-third of the total elimination [32]. However, we do not find it justified to calculate any extrahepatic elimination of ethanol from the present high-concentration periods because the approximation to steady-state cannot be evaluated exactly enough. Thus, despite the high hepatic extraction fractions in the present low-concentration periods, being 0.73–1.00 in the patients with cirrhosis and 0.98–1.00 in the healthy subjects, the substantial extrahepatic elimination precludes the use of the systemic clearance of ethanol, equal to the ethanol infusion rate divided by arterial concentration, as a measure of liver blood flow in humans [22].

In summary, the present *in vivo* study of hepatic elimination kinetics of ethanol in humans shows that in patients with cirrhosis, neither V_{max} nor K_m is significantly different from the values in healthy subjects. The kinetic analysis suggests that the hepatic ethanol elimination is governed primarily by the cytosolic ADH system in both groups of subjects, in agreement with previous *in vivo* studies in rats and humans. The results are interpreted as evidence

against a significant contribution of microsomal ethanol oxidation compared to that of ADH *in vivo* in patients with alcoholic cirrhosis as well as in healthy subjects.

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References

- [1]. Lundquist F, Tygstrup N, Winkler K, Mellemegaard K, Munck-Petersen S. Ethanol metabolism and production of free acetate in the human liver. *J Clin Invest.* 1962; 41:955–61. [PubMed: 14467395]
- [2]. Wartburg JP, Bethune JL, Vallee BL. Human liver-alcohol dehydrogenase. Kinetic and physicochemical properties. *Biochemistry.* 1964; 3:1775–82. [PubMed: 14235347]
- [3]. Crabb DW, Bosron WF, Li TK. Steady-state kinetic properties of purified rat liver alcohol dehydrogenase: application to predicting alcohol elimination rates *in vivo*. *Arch Biochem Biophys.* 1983; 224:299–309. [PubMed: 6347067]
- [4]. Ehrig T, Bosron WF, Li TK. Alcohol and aldehyde dehydrogenase. *Alcohol Alcoholism.* 1990; 25:105–16.
- [5]. Lieber CS, DeCarli LM. Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science.* 1968; 162:917–8. [PubMed: 4386718]
- [6]. Lieber CS, DeCarli LM. Hepatic microsomal ethanoloxidizing system. *In vitro* characteristics and adaptive properties *in vivo*. *J Biol Chem.* 1970; 245:2905–12.
- [7]. Asai H, Imaoka S, Kuroki T, Monna T, Funae Y. Microsomal ethanol oxidizing system activity by human hepatic cytochrome P450s. *J Pharm Exp Ther.* 1996; 277:1004–9.
- [8]. Lieber CS, DeCarli LM. The role of hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism *in vivo*. *J Pharm Exp Ther.* 1972; 181:279–87.
- [9]. Teschke R, Hasumura Y, Lieber CS. Hepatic microsomal alcohol-oxidizing system. Affinity for methanol, ethanol, propanol, and butanol. *J Biol Chem.* 1975; 250:7397–404. [PubMed: 240827]
- [10]. Kalant H, Khanna JM, Endrenyi L. Effect of pyrazole on ethanol metabolism in ethanol-tolerant rats. *Can J Physiol Pharmacol.* 1975; 53:416–22. [PubMed: 1148928]
- [11]. Khanna JM, Kalant H. *In vivo* significance of the microsomal ethanol oxidizing system (MEOS). *Adv Exp Med Biol.* 1977; 85A:281–302. [PubMed: 200118]
- [12]. Keiding S, Christensen NJ, Damgaard SE, Dejgård A, Iversen HL, Jacobsen A, et al. Ethanol metabolism in heavy drinkers after massive and moderate alcohol intake. *Biochem Pharmacol.* 1983; 32:3097–102. [PubMed: 6639677]
- [13]. Crabb DW, Bosron WF, Li T-K. Ethanol metabolism [review]. *Pharmacol Ther.* 1987; 34:59–73. [PubMed: 3310044]
- [14]. Norberg Å, Jones AW, Hahn RG, Gabrielsson JL. Role of variability in explaining ethanol pharmacokinetics; research and forensic applications [review]. *Clin Pharmacokinet.* 2003; 42:1–31. [PubMed: 12489977]
- [15]. Jones AW. Ultra-rapid rate of ethanol elimination from blood in drunken drivers with extremely high blood-alcohol concentrations. *Int J Legal Med.* 2008; 122:129–34. [PubMed: 17572905]
- [16]. Teschke R, Gellert J. Hepatic microsomal ethanol-oxidizing system (MEOS): metabolic aspects and clinical implications [review]. *Alcoholism: Clin Exp Res.* 1986; 10(Suppl6):20S–32S.
- [17]. Lieber CS. The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. *Drug Metab Rev.* 2004; 36:511–29. [PubMed: 15554233]
- [18]. Mello T, Ceni E, Surrenti C, Galli A. Alcohol-induced hepatic fibrosis: role of acetaldehyde [review]. *Mol Aspects Med.* 2008; 29:17–21. [PubMed: 18164754]
- [19]. Keiding S, Johansen S, Midtbøll I, Rabøl A, Christiansen L. Ethanol elimination kinetics in human liver and pig liver *in vivo*. *Am J Physiol.* 1979; 237:E316–24. [PubMed: 495711]
- [20]. Bass L, Keiding S, Winkler K, Tygstrup N. Enzymatic elimination of substrates flowing through the intact liver. *J Theor Biol.* 1976; 61:393–409. [PubMed: 979305]

- [21]. Keiding S, Johansen S, Winkler K, Tønnesen K, Tygstrup N. Michaelis–Menten kinetics of galactose elimination by the isolated perfused pig liver. *Am J Physiol.* 1976; 320:1302–13. [PubMed: 1275072]
- [22]. Keiding, S.; Sørensen, M. Hepatic removal kinetics: importance for quantitative measurements of liver function. In: Rodes, J.; Benhamou, J-P.; Dufour, JF.; Blei, A.; Reichen, J.; Rizzetto, M., editors. *Textbook of hepatology: from basic science to clinical practice.* 3rd edn.. Blackwell; Oxford: 2007. p. 561–6.
- [23]. Greenway CV, Lauth WW, Sitar DS. Hepatic blood flow: Estimation of clearances of very low dose infusions of ethanol in cats. *Can J Physiol Pharmacol.* 1988; 66:1191–7.
- [24]. Winkler K, Bass L, Keiding S, Tygstrup N. Physiologic basis of clearance measurements in hepatology [review]. *Scand J Gastroenterol.* 1979; 14:439–48. [PubMed: 482856]
- [25]. Tygstrup N. The galactose elimination capacity in relation to clinical and laboratory findings in patients with cirrhosis. *Acta Med Scand.* 1964; 175:290–300.
- [26]. Tygstrup N. Effect of sites of blood sampling in determination of the galactose elimination capacity. *Scand J Clin Lab Invest.* 1977; 18:333–8. [PubMed: 616060]
- [27]. Keiding S, Vilstrup H. Intrahepatic heterogeneity of hepatic venous pressure gradient in human cirrhosis. *Scand J Gastroenterol.* 2002; 37:960–4. [PubMed: 12229973]
- [28]. Skak C, Keiding S. Methodological problems in the use of indocyanine green to estimate hepatic blood flow and ICG clearance in man. *Liver.* 1987; 7:155–62. [PubMed: 3613884]
- [29]. Nielsen NC. Correction for blank density caused by turbidity in human plasma. *Scand J Clin Lab Invest.* 1965; 17:165–70. [PubMed: 14285924]
- [30]. Nielsen NC. Spectrophotometric determination of indocyanine green in plasma especially with a view to an improved correction for blank density. *Scand J Clin Lab Invest.* 1963; 15:613–21. [PubMed: 14077016]
- [31]. Lundquist, F. The determination of ethylalcohol in blood and tissues. In: Glück, D., editor. *Methods of biochemical analyses.* Interscience; New York: 1959. p. 217–51.
- [32]. Utne HE, Winkler K. Hepatic and extra-hepatic elimination of ethanol in cirrhosis with estimates of intra-hepatic shunts and K_m for ethanol elimination. *Scand J Gastroenterol.* 1980; 15:297–304. [PubMed: 7433889]
- [33]. Buris L, Csabai G, Fodor M, Varga M. Increase in alcohol dehydrogenase and protein content of liver following chronic ethanol administration. *FEBS Lett.* 1985; 183:143–4. [PubMed: 3156763]
- [34]. Holund B, Poulsen H, Schlichting P. Reproducibility of liver biopsy diagnosis in relation to the size of the specimen. *Scand J Gastroenterol.* 1980; 155:329–35. [PubMed: 7433892]
- [35]. Bass L, Robinson P, Bracken AJ. Hepatic elimination of flowing substrates: the distributed model. *J Theor Biol.* 1978; 72:161–84. [PubMed: 661331]
- [36]. Winkler K, Lundquist F, Tygstrup N. The hepatic metabolism of ethanol in patients with cirrhosis of the liver. *Scand J Clin Lab Invest.* 1969; 23:59–69. [PubMed: 5363436]

Figure 1. Blood concentrations of ethanol in an artery (●) and a liver vein (○) in a patient with cirrhosis (no. 5) during 5 periods of successive ethanol infusions in increasing doses of 0.45, 0.89, 1.23, 1.51 and 1.90 mmol min⁻¹.

Table 1

Hepatic ethanol elimination kinetics in humans

Subject no.	Gender/age (M;F/years)	GEC (mmol min ⁻¹)	Ethanol V _{max} (mmol min ⁻¹)	Ethanol K _m (mM)
Patients with cirrhosis				
1	M/51	1.53	1.85	0.24
2	M/70	1.78	1.71	0.16
3	M//61	1.15	1.95	0.69
4	F/63	0.98	1.04	0.16
5	M/72	1.10	1.32	0.63
ID3 in [19]	F/44	ND	1.38	0.26
Mean±SEM		1.31±0.04	1.54±0.14	0.36±0.11
Healthy subjects				
7	M/24	1.92	2.09	0.36
8	M/25	1.76	1.21	0.14
9	M/29	1.94	1.43	0.13
10	M/20	2.15	1.28	0.12
ID1 in [19]	F/44	ND	1.12	0.09
ID2 in [19]	F/49	ND	1.36	0.13
Mean±SEM		1.94±0.08 [*]	1.42±0.14 [#]	0.16±0.04 [#]

Abbreviations: M = male; F = female; ND = not done; SEM = standard error of the mean; GEC = galactose elimination capacity, a measure of V_{max} for liver cytosolic galactokinase.

* $p < 0.01$ versus healthy subjects

$p > 0.3$ versus healthy subjects.