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ORIGINAL ARTICLE

Inhibition of 1,4-butanediol metabolism in human liver in vitro

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Abstract The conversion of 1,4-butanediol (1,4-BD) to gamma-hydroxybutyric acid (GHB), a drug of abuse, is most probably catalyzed by alcohol dehydrogenase, and potentially by aldehyde dehydrogenase. The purpose of this study was to investigate the degradation of 1,4-BD in cytosolic supernatant of human liver in vitro, and to verify involvement of the suggested enzymes by means of gas chromatography–mass spectrometry. The coingestion of 1,4-BD and ethanol (EtOH) might cause complex pharmacokinetic interactions in humans. Therefore, the effect of EtOH on 1,4-BD metabolism by human liver was examined in vitro. Additionally, the influence of acetaldehyde (AL), which might inhibit the second step of 1,4-BD degradation, was investigated. In case of a 1,4-BD intoxication, the alcohol dehydrogenase inhibitor fomepizole (4-methylpyrazole, FOM) has been discussed as an antidote preventing the formation of the central nervous system depressing GHB. Besides FOM, we tested pyrazole, disulfiram, and cimetidine as possible inhibitors of the formation of GHB from 1,4-BD catalyzed by human liver enzymes in vitro. The conversion of 1,4-BD to GHB was inhibited competitively by EtOH with an apparent K_i of 0.56 mM. Therefore, the coingestion of 1,4-BD and EtOH might increase the concentrations and the effects of 1,4-BD itself. By contrast AL accelerated the formation of GHB. All antidotes showed the ability to inhibit the formation of GHB. In comparison FOM showed the highest inhibitory effectiveness. Furthermore, the results confirm strong involvement of ADH in 1,4-BD metabolism by *human* liver.

Keywords 1,4-Butanediol · Gamma-hydroxybutyric acid · GHB · Liquid ecstasy · Metabolism · Human liver enzymes · Alcohol dehydrogenase · Aldehyde dehydrogenase

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Introduction

Gamma-hydroxybutyric acid (GHB) is abused in doses of 20–30 mg/kg as a recreational drug (Couper and Marinetti 2002). Therefore, GHB is known as liquid ecstasy. If higher amounts of this drug are ingested (≥50 mg/kg) it may cause unconsciousness and coma, leading to its misuse as a date rape drug at drug-facilitated sexual assaults. Therapeutically, the GHB sodium salt is used as anesthetic and for treatment of narcolepsy and alcohol craving. Unlike GHB its metabolic precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) are not ranked as narcotic substances in many countries. Numerous lethal intoxications caused either by intake of 1,4-BD, GBL, or GHB itself have been reported (Kintz et al. 2005; Lenz et al. 2008; Lora-Tamayo et al. 2003; Brent 2009).

After ingestion, 1,4-BD is metabolized to gamma-hydroxybutyraldehyde and finally to GHB in a double-stage, nicotinamide adenine dinucleotide (NAD)-dependent reaction. It was shown that this reaction is most probably catalyzed by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) using purified horse liver ADH and cytosolic preparations from different rat organs containing both ADH and ALDH (Fig. 1) (Bessman and McCabe 1972; Poldrugo and Snead 1986).

Ethanol (EtOH) is another substrate for ADH. After administration of both EtOH and 1,4-BD to rats, increased

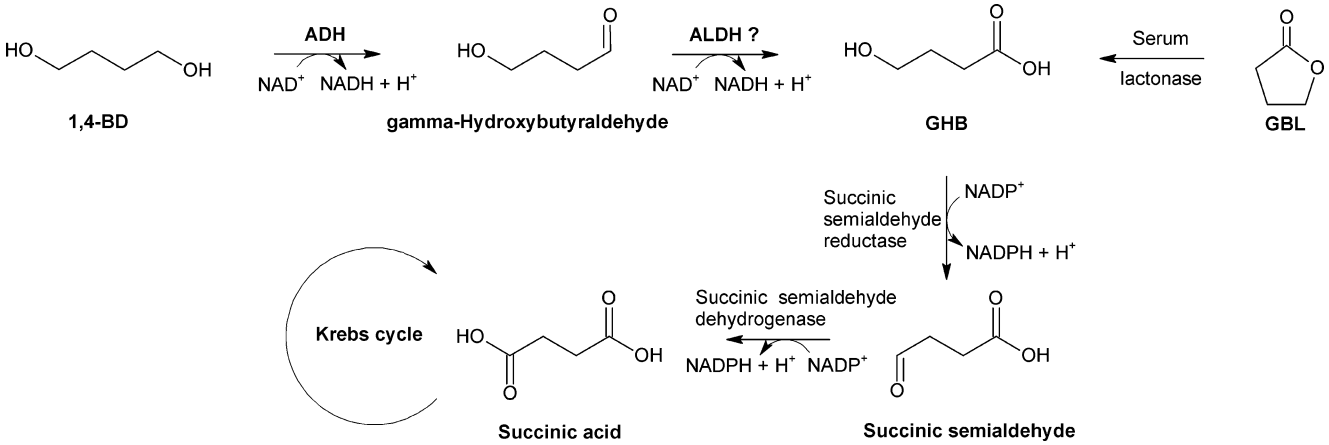

Fig. 1 Putative metabolic pathway of 1,4-butanediol (*1,4-BD*) and gamma-butyrolactone (*GBL*) in mammals: oxidation of 1,4-BD by alcohol dehydrogenase (*ADH*) to gamma-hydroxybutyraldehyde, which is converted to gamma-hydroxybutyric acid (*GHB*), potentially by aldehyde dehydrogenase (*ALDH*). The hydrolysis of GBL is catalyzed by serum lactonase. GHB is degraded by succinic semialdehyde reductase. The resultant succinic semialdehyde undergoes metabolism to succinic acid catalyzed by succinic semialdehyde dehydrogenase. Both steps of GHB metabolism are nicotinamide adenine dinucleotide phosphate (*NADP*)-dependent reactions

plasma and organ concentrations of 1,4-BD were observed compared to a single administration of 1,4-BD (Fung et al. 2008; Poldrugo et al. 1985; Poldrugo and Snead 1986). The increase of 1,4-BD concentrations may be caused by a competition of EtOH and 1,4-BD for degradation by ADH, whereas EtOH has a somewhat higher affinity to rat liver ADH compared to 1,4-BD ($K_{m\ 1,4\text{-BD}}$, 0.75 mM; $K_{m\ EtOH}$, 0.16–0.5 mM) (Fung et al. 2008; Lad and Leffert 1983; Poldrugo and Snead 1986; Reynier 1969; Zorzano and Herrera 1989). Seventy-five minute after administration of both drugs the 1,4-BD levels in brain, liver, and kidney increased significantly at least by factor 3.5 compared to animals receiving 1,4-BD only (Poldrugo et al. 1985). Furthermore, an increased mortality of rats was observed after administration of both (EtOH+1,4-BD, 50% mortality) 1,4-BD (1 g/kg) and EtOH (3 g/kg) in comparison to a single administration of each drug (EtOH, 0% mortality; 1,4-BD, 6% mortality). As cause for the high mortality both raised 1,4-BD levels reaching the LC_{50} and the summation of the effects of 1,4-BD and EtOH were discussed (Poldrugo et al. 1985). Considering a supposable consumption of 1,4-BD with EtOH as recreational drugs these results are alarming (Snead and Gibson 2005). It was observed that the blood EtOH concentration of rats was not affected by administration of both drugs (Poldrugo and Snead 1984; Poldrugo et al. 1985). In contrast to the findings of Poldrugo and Snead a population pharmakokinetic model, which is based on in vivo experiments with rats, showed mutual inhibition of metabolism between 1,4-BD and EtOH (Fung et al. 2008). Until now, the in vitro metabolism of 1,4-BD as well as the inhibition of this pathway by EtOH have never been investigated with human enzymes or tissue extracts.

The other toxicological issue of 1,4-BD is caused by its degradation product GHB and may induce serious effects as well. Among them, coma and severe respiratory depression are most prominent. Until now, the only remedy for GHB intoxication is a supportive management of vital functions. However, in case of 1,4-BD intoxications the inhibition of ADH or ALDH is a potential target in order to prevent further formation of the central depressing GHB. The prevention of 1,4-BD toxicity by blocking the conversion to GHB has been discussed before (Carai et al. 2002; Carai et al. 2006; Dempsey et al. 2009; Megarbane et al. 2002). Carai et al. showed that mice receiving a lethal amount of 1,4-BD were resuscitated by a timely application of fomepizole (FOM) (Carai et al. 2006). This suggests the benefit of the inhibition of 1,4-BD metabolism in case of a 1,4-BD intoxication. However, until now a risk–benefit assessment concerning this issue has not been investigated in humans, whereas the prolonged effect of 1,4-BD itself must be taken into account especially.

In this study the effect of EtOH on 1,4-BD metabolism by human liver enzymes was investigated in order to evaluate the pharmacokinetic interaction. Besides EtOH, the EtOH metabolite acetaldehyde (AL) was employed as an inhibitor as well, in order to evaluate the interaction on the second stage of this metabolic pathway. The second purpose was to investigate the in vitro efficiency of potential antidotes for a 1,4-BD intoxication which may inhibit the GHB formation. Beside the potent ADH inhibitors fomepizole and pyrazole (PYR) the ALDH inhibitor disulfiram (DSF) and cimetidine (CIM), an H_2-antihistaminic with a putative ADH-inhibiting property, were tested by determination of their half-maximal inhibitory concentration (IC_{50}). These inhibitors were chosen due to

their comparatively low toxicity, well-known pharmacological properties, and, except for CIM and PYR, their clinical approval (Brent 2009; Carai et al. 2002; Dawidek-Pietryka et al. 1998). CIM is approved for the inhibition of gastric acid secretion.

Material and methods

Chemicals and reagents

1,4-BD (≥99%), 1,5-pentanediol (1,5-PD, ≥97%), GHB sodium salt, FOM (99%), cimetidine, pyrazole (98%), and disulfiram (≥99%, DSF) were purchased from Sigma Aldrich (Steinheim, Germany). Nicotinamide adenine dinucleotide (free acid, gradient grade I, 100%) was obtained from Roche Diagnostics (Mannheim, Germany). The methanolic solution of sixfold deuterated GHB sodium salt (GHBd6; 1 mg/ml) was delivered by LGC Promochem (Wesel, Germany). Ethanol (≥99%, EtOH), water (ROTISOLV, gradient grade), di-sodium hydrogen phosphate anhydrous (≥99%, p.a.), and potassium dihydrogen phosphate (≥99%, p.a.) were acquired from Carl Roth (Karlsruhe, Germany). Acetonitrile (gradient grade) and methanol (LiChrosolv, gradient grade) were purchased from Merck (Darmstadt, Germany). The silylation agent *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) was obtained by Macherey-Nagel (Düren, Germany). The Uptima BC assay protein quantitation kit was from Interchim (Montluçon, France).

Cytosolic supernatant of human livers

Samples of ten human livers (five female and five male donors; age, 43–79 years) were collected during autopsy within 72 h after death and were stored at −80°C until preparation of the pooled supernatants. The records of each case document that there was no obvious influence (e.g., by medication or liver disease) on the relevant enzymatic system. Immediately after defrosting, the liver samples were homogenized in ice-cold 100 mM phosphate buffer (pH 7.4) using a blender (ratio 1 g–3 ml). The homogenates were centrifuged at 15,000×*g* for 30 min at 2°C. Afterwards all homogenates were pooled. The obtained extract was centrifuged at 120,000×*g* for 45 min at 2°C. The pooled supernatant was aliquoted in 1.5 ml Eppendorf cups and stored at −80°C until utilization. The photometric determination of the total protein concentration was performed with a protein quantitation kit, which is based on a method using bicinchoninic acid (Smith et al. 1985). The activity of the relevant enzymes depends on both the postmortem interval and storage conditions. Although the donor bodies did not show any relevant decomposition, a partial inactivation of enzymes cannot be excluded. Furthermore, interindividual differences of the activity of ADH or ALDH due to, e.g., polymorphisms must be taken into account as well.

Ethical concern

This study has been performed in accordance with the declaration of Helsinki and has been approved by the ethics committee of the University of Cologne. All relatives were informed about the purpose of this study and gave written consent.

Incubations

All incubations were performed in 1.5 ml Eppendorf cups. For the enzymatic degradation of 1,4-BD to GHB the incubation time and concentration of both cofactor (nicotinamide adenine dinucleotide) and protein were optimized. The incubation experiments were performed with a thermoshaker (Eppendorf, Hamburg, Germany) at 37°C and 800 rpm. The final reaction mixtures contained 6.25 µl cytosolic supernatant and enough buffer to fill the volume to 250 µl. The final NAD concentration was 5 mM. The reaction was started by addition of cytosolic supernatant. Unless specified otherwise, stock solutions of cofactor, substrate, and inhibitors were prepared with 100 mM phosphate buffer (pH 7.4). The final protein concentration and incubation time were 525 µg/ml and 20 min, respectively, which were in the linear range of GHB formation for each parameter.

The inhibition of 1,4-BD metabolism at substrate concentrations of 3, 5, 10, 20, 40, and 80 mM by EtOH was investigated without inhibitor and at three different EtOH concentrations (0.5, 1, and 2 mM EtOH). Additionally, acetaldehyde was employed as inhibitor (0.5, 1, and 2 mM AL). Here the following 1,4-BD concentrations were used: 1, 2, 3, 5, 10, 20, 40, and 80 mM. All incubations were performed fivefold. Calibrators were prepared without cofactor but with cytosolic supernatant as matrix.

The IC_{50} of potential antidotes were determined at three different substrate levels (0.5, 2, and 5 mM 1,4-BD). The 1,4-BD concentrations were chosen according to the determined Michaelis–Menten constant (K_m) and to published 1,4-BD concentrations listed in case reports (Lora-Tamayo et al. 2003; Megarbane et al. 2002; Duer et al. 2001; Zvosec et al. 2001). Apart from controls (without inhibitor, 100% activity) incubations were performed with individual ranges of inhibitor concentrations (FOM, 0.01–10,000 µM; PYR, 0.1–100,000 µM; CIM, 25–10,000 µM; DSF, 2.5–1,000 µM). DSF stock solutions were prepared in acetonitrile, since this agent exhibits insufficient water solubility. Here, the acetonitrile content in the assay mixture was 2% [*v*/*v*]. In this case the control (without DSF) contained 2% [*v*/*v*] acetonitrile as well. Here, all incuba-

tions were assayed threefold and the incubation time was 60 min. In additional experiments both the degradation of 5 mM 1,4-BD and the formation of GHB were investigated simultaneously. Here somewhat different conditions were chosen. Briefly, besides a higher protein concentration (17.0 mg/ml) a prolonged incubation (0–240 min) was applied. 1,5-PD was used as internal standard for determination of 1,4-BD.

The reaction was stopped by adding 1,000 µl of an acetonitrile/methanol mixture (95:5 [*v*/*v*]) containing GHBd6 and 1,5-PD as internal standards to 200 µl of the reaction mixture. Calibrators were stopped immediately after preparation. The mixture was centrifuged at 21,100×*g* for 5 min. Afterwards 1,000 µl of the supernatants were evaporated to dryness at 40°C under a nitrogen stream. The residue was reconstituted in 100 µl MSTFA and derivatized at 60°C for 20 min. One microliter of each sample or calibrator was injected into the gas chromatography–mass spectrometry (GC-MS) instrument.

Gas chromatography–mass spectrometry

A Thermo Trace GC ultra gas chromatograph—coupled with a Polaris Q ion trap mass spectrometer—was used for analysis (Thermo Fisher Scientific, Dreieich, Germany). The injection was performed splitless. The GC-MS system was equipped with a J&W HP-1ms GC-capillary column (100% polydimethylsiloxane; 15 m×0.25 mm×0.25 µm; Agilent Technologies, Waldbronn, Germany). The temperatures of the injector, transferline, and ion source were set to 280°C, 290°C, and 250°C respectively. Helium 4.6 was used as carrier gas with a flow rate of 1.0 ml/min. The following temperature program was applied: 1 min at 60°C, 10°C/min to 130°C, 40°C/min to 290°C, held for 1 min. The mass spectrometer was operated with electron impact ionization at 70 eV in segmented scan mode (mass ranges, *m*/*z* 114–120 and *m*/*z* 201–242). The electron multiplier was set to 1,550 V. For the detection of GHB- and GHBd6-di-trimethylsilyl derivatives, the following fragments were used: *m*/*z* 117, 204, and 233 for GHB (GHB-di-TMS); *m*/*z* 119, 206, and 239 for GHBd6 (GHBd6-di-TMS). Peak area ratios of *m*/*z* 233 and 239 were used for quantitation (Fig. 2). All steps such as data acquisition, processing, and evaluation were conducted with the instrument software Xcalibur 1.3. The accuracy (bias) determined at GHB concentrations of 10, 100, and 500 µM was 16.7%, −3.6%, and 2.6%. The interday precision (relative standard deviation) was 9.9%, 3.1%, and 2.9%, respectively.

For detection of di-trimethylsilyl-derivatives of 1,4-BD (1,4-BD-di-TMS; *m*/*z* 177 and 219) and 1,5-PD (1,5-PD-di-TMS; *m*/*z* 177 and 233) besides GHB and GHBd6, the

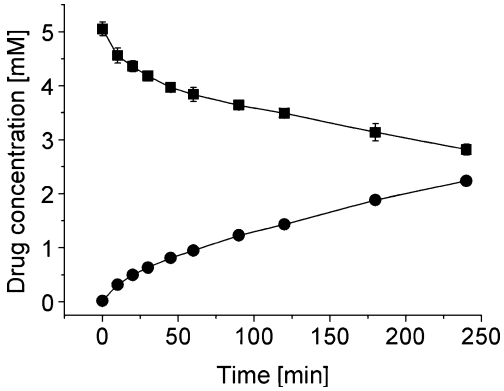

Fig. 3 Degradation of 1,4-BD (*filled square*) at an initial concentration of 5 mM and formation of GHB (*filled circle*) in cytosolic supernatant of human liver over a period of 240 min. Drug concentrations are given as mean ± standard deviation, (*n*=5). *Error bars* are often smaller than the symbol

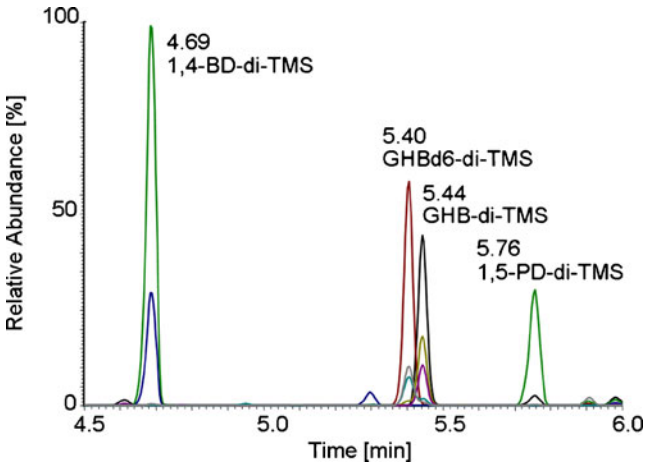

Fig. 2 Chromatogram of an incubation mixture showing the ion traces of 1,4-BD (*m*/*z* 177 and 219), GHBd6 (*m*/*z* 119, 206, and 239), GHB (*m*/*z* 117, 204, and 233), and 1,5-PD-di-TMS (*m*/*z* 177 and 233)

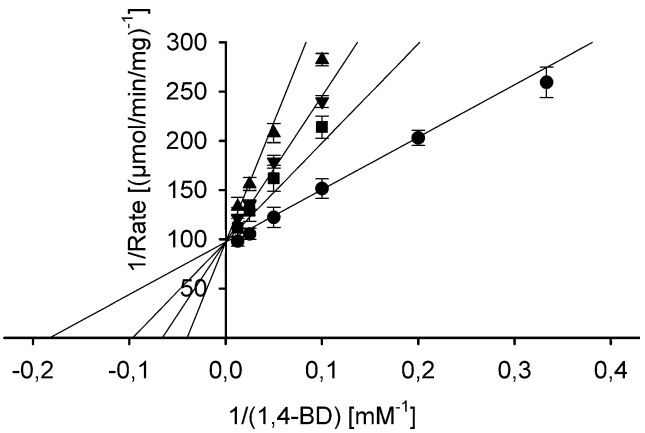

Fig. 4 Lineweaver–Burk plot showing the effect of ethanol on 1,4-BD metabolism by cytosolic supernatant of human liver in vitro: no inhibitor (*filled circle*), 0.5 mM (*filled square*), 1 mM (*filled inverted triangle*), and 2 mM ethanol (*filled triangle*). 1/Rate [(micromoles per minute per milligram of protein)$^{-1}$] is given as mean ± standard deviation, (*n*=5)

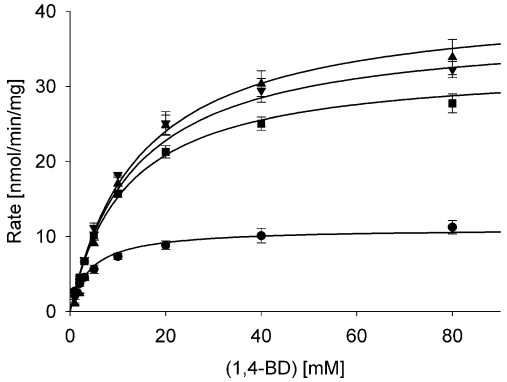

Fig. 5 Michaelis–Menten plot of gamma-hydroxybutyric acid formation from 1,4-butanediol at different AL levels in vitro: 0 mM (*filled circle*), 0.5 mM (*filled square*), 1 mM (*filled inverted triangle*), and 2 mM AL (*filled triangle*)

same temperature program was applied in full scan mode (*m*/*z* 170–240).

Calculation of pharmacological parameters and data analysis

The enzyme kinetic parameters K_m, inhibition constant (K_i), and maximum rate (V_{max}) were calculated using a nonlinear regression analysis program (SigmaPlot Enzyme Kinetics Module 1.1, SigmaPlot software, Chicago, IL, USA). The IC_{50} values were calculated according to a sigmoidal logistic four-parametric regression model using the Origin 8.1 software (OriginLab Corporation, Northampton, MA, USA). The statistical significance ($p<0.05$) of the differences was evaluated using a *t* test.

Results

Preliminary results showed that GHB is stable in the incubation mixture, which was assayed for a period of 120 min. Further investigations showed that GHB is formed almost as fast as the initial amount of the substrate is decreased in an in vitro assay with a high protein content (Fig. 3).

EtOH inhibited the conversion of 1,4-BD to GHB at different concentrations (3–80 mM). The enzyme kinetic data were fitted to a competitive inhibition model by a nonlinear regression analysis yielding a sufficient correlation ($R^2=0.965$; Fig. 4).

The apparent K_m, V_{max}, and K_i were 5.5 mM, 10.0 nmol/min/mg, and 0.56 mM, respectively. Unlike EtOH its metabolite AL did not inhibit 1,4-BD degradation in vitro. Surprisingly, an increased formation of GHB was observed here, which is visualized by the Michaelis–Menten plot shown in Fig. 5.

At AL levels of 0.5, 1, and 2 mM V_{max} was increased by factor 2.8, 3.4, and 3.8, respectively. The Scatchard plot of these data revealed a parabolic curve at a constant AL level (2 mM) as shown in Fig. 6b (Hill coefficient, 1.4).

All tested antidotes inhibited the metabolism of 1,4-BD to GHB in cytosolic supernatant of human liver in vitro (Fig. 7).

The IC_{50} of the tested drugs are summarized in Table 1. In all used substrate concentrations, FOM was the most potent inhibitor. This was significant compared to PYR and DSF at all tested concentrations. PYR was less potent than FOM, but showed an almost complete inhibition of GHB formation from 1,4-BD at high concentrations like FOM.

Due to its limited water solubility, CIM was not used at concentrations higher than 10 mM in the assay. Here a saturation of its effects was not reached. Therefore, the determination of its IC_{50} was performed approximatively. CIM was the weakest inhibitor by far. DSF showed an incomplete inhibition, with about 40% residual metabolic activity after saturation of its effects. Acetonitrile, which was used as solvent for DSF, caused a decrease of the enzymatic activity of about 30% at a concentration of 2% [*v*/*v*].

Discussion

The results show that the metabolism of 1,4-BD is inhibited by EtOH in cytosolic supernatant of human liver in vitro, which confirms the thesis of potential pharmacokinetic interaction of both drugs in human. Dempsey et al.

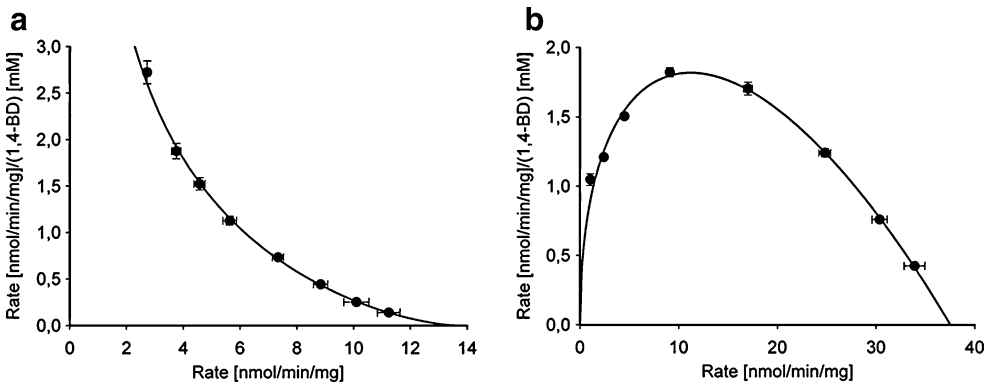

Fig. 6 Scatchard plots of gamma-hydroxybutyric acid formation from 1,4-butanediol in vitro: **a** without acetaldehyde, **b** parabolic curve progression in the presence of acetaldehyde (2 mM), mean ± standard deviation ($n=5$)

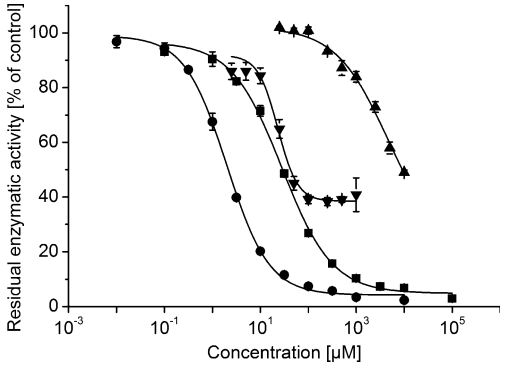

Fig. 7 Inhibition of GHB formation from 1,4-BD (0.5 mM) in cytosolic supernatant of human liver in vitro, given as residual enzymatic activity ± standard error of mean, (n=3): fomepizole (*filled circle*), pyrazole (*filled square*), disulfiram (*filled inverted triangle*), and cimetidine (*filled triangle*)

demonstrated involvement of ADH in human 1,4-BD metabolism (Dempsey et al. 2009).

FOM is a strong and specific competitive inhibitor of ADH (Theorell and Yonetani 1969). Due to the almost complete inhibition of GHB formation caused by FOM, our results suggest that ADH is the main enzyme for 1,4-BD degradation in our experiments and most probably in human. Our results showed that GHB is formed almost as fast as 1,4-BD is degraded. This suggests that the first step of 1,4-BD metabolism is rate limiting. This issue is known for EtOH metabolism as well, on the condition that there is no ALDH deficiency (Eriksson 1983; Tsukamoto et al. 1989). Therefore, and according to Poldrugo et al., it can be concluded that the estimated enzyme kinetic parameters represent the kinetic of the first step of 1,4-BD metabolism catalyzed by ADH (Poldrugo and Snead 1986).

The 1,4-BD degradation to GHB in cytosolic rat liver extract was decreased by DSF (Poldrugo and Snead 1986). An incomplete antagonism of the sedative effects (sleep time, onset of sleep) mediated by 1,4-BD was observed in mice pretreated with DSF (Carai et al. 2002). In our study, DSF inhibited the GHB formation up to 60%. Therefore our results confirm the involvement of another enzyme, which is responsible for the degradation of gamma-hydroxybutyraldehyde (Poldrugo and Snead 1986). Another explanation for the incomplete inhibition by DSF might be a delayed onset of inhibition, since the mechanism of DSF seems to be based on the formation of an intramolecular disulfide bond. This was supported by experiments with rat liver mitochondrial ALDH, whereas the inhibition of this enzyme was time dependent with 42% of initial activity after 10 min of incubation with DSF (Shen et al. 2000). Fifty minutes later about 16% of ALDH activity were left.

Table 1 Comparison of IC_{50} of tested antidotes

1,4-BD concentration, [mM]	IC_{50} ± standard error of mean (n=3), [µM]			
	Fomepizole	Pyrazole	Disulfiram	Cimetidine
0.5	2.0±0.2	28.0±0.8*	21.5±2.6*	4,643±650*
2	3.6±0.1	60.7±2.7*	22.8±1.2*	4,795±1,369
5	6.2±0.2	120.0±4.5*	25.7±2.7*	4,002±395*

*p<0.05, statistically significant difference compared to fomepizole

The GHB formation from 1,4-BD was accelerated by AL in rat liver extract (Poldrugo and Snead 1986). The authors expected an inhibition of 1,4-BD metabolism due to a competition of AL and gamma-hydroxybutyraldehyde for degradation by ALDH. A further degradation of the formed GHB in the assay was not ruled out by the authors. Poldrugo et al. discussed an inhibition of GHB degradation by AL, whereby the GHB concentrations could have been increased compared to incubations without AL.

In the present study, AL caused an acceleration of 1,4-BD metabolism to GHB in human liver as well. The explanation suggested by Poldrugo et al. can be excluded here, because there was no degradation of GHB in the incubation mixture for at least 120 min (data not shown). Obviously, the enzymes being responsible for GHB metabolism were not active here. Our findings suggest that the increased formation of GHB from 1,4-BD might be caused by a positive cooperativity at the dimeric ADH where AL acts as modulator. This is visualized by the Michaelis–Menten plot in Fig. 5. Interestingly, the Scatchard plot at a constant AL level (2 mM) reveals a parabolic curve progression shown in Fig. 6b, which suggests the involvement of 1,4-BD in this interaction as well. If AL is not present, the Scatchard plot suggests absence of cooperativity or positive allosteric regulation, as shown in Fig. 6a. Further investigations are necessary for the elucidation of this phenomenon. However, this issue seems to be irrelevant concerning the pharmacokinetic interaction of 1,4-BD and EtOH, because an increase of GHB formation was not observed if both drugs were incubated. Furthermore, an in vivo accumulation of AL, which could theoretically increase GHB formation, seems unlikely due to the fast degradation of AL (Eriksson 1983; Tsukamoto et al. 1989). If an increase of total reaction rate was observed, the slower of both reactions must have been accelerated here. The reaction catalyzed by ADH is obviously rate limiting. A cooperativity at ALDH, which catalyzes the oxidation of gamma-hydroxybutyraldehyde, appears to be unlikely since this would most probably not cause an increase of the total reaction rate.

FOM was applied to six probands before administration of a low dose of 1,4-BD (10 mg/kg) in order to prevent GHB formation (Dempsey et al. 2009). Although FOM decreased

GHB formation, no significant differences of the effects (vital signs, oxygen saturation, and subjective effects) caused by 1,4-BD (control group) or 1,4-BD preceded by FOM administration were observed. However, the rescuing effect of a timely FOM administration was investigated in mice, which received a lethal dose of 1,4-BD before (Carai et al. 2006). Even 90 min after 1,4-BD administration all animals were resuscitated by 30 mg FOM/kg. In another study, the complete blocking of the sedative effects of 1,4-BD was observed in mice pretreated with FOM (Carai et al. 2002). If FOM was administered to mice after receiving 1,4-BD, a shorter impairment of their performance was observed (Quang et al. 2004). Finally, a man intoxicated with 1,4-BD, causing generalized seizures and coma, received treatment with FOM (Megarbane et al. 2002). He recovered within 3 h, although it is unknown whether FOM influenced the clinical course. Our results show that among the tested antidotes FOM is most effective in inhibiting GHB formation from 1,4-BD by cytosolic supernatant in vitro. Therefore, FOM is suggested as the most suitable antidote to counter GHB effects caused by an acute 1,4-BD intoxication. CIM, which inhibited the methanol metabolism by human ADH in vitro more effectively than FOM (Dawidek-Pietryka et al. 1998), exhibited the lowest inhibitory efficacy in our study. The determined IC_{50} of DSF and CIM appear to be independent of the substrate concentration, since the inhibition cannot be overcome by increasing the substrate concentration. This issue suggests a non-competitive inhibition for DSF and CIM in our study.

The inhibition of 1,4-BD metabolism appears reasonable in order to block the formation of central depressing GHB in case of an acute 1,4-BD intoxication. However, a thorough risk–benefit assessment is necessary concerning the supposable increase of 1,4-BD levels after inhibition of ADH. Especially in case of a 1,4-BD and EtOH overdose this approach might cause an unpredictable increase of both 1,4-BD and EtOH levels. This should be considered thoroughly in each individual case. EtOH should not be used as an ADH inhibitor regarding the potentially dangerous interactions between EtOH and 1,4-BD (Poldrugo and Snead 1984; Poldrugo et al. 1985).

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Conflict of interest The authors declare that they have no conflict of interest.

References

Bessman SP, McCabe RB (1972) 1,4-butanediol—substrate for rat-liver and horse liver alcohol dehydrogenases. Biochem Pharmacol 21 (8):1135–1142

Brent J (2009) Fomepizole for ethylene glycol and methanol poisoning. N Engl J Med 360(21):2216–2223

Carai MAM, Colombo G, Reali R, Serra S, Mocci I, Castelli MP, Cignarella G, Gessa GL (2002) Central effects of 1,4-butanediol are mediated by GABA(B) receptors via its conversion into gamma-hydroxybutyric acid. Eur J Pharmacol 441(3):157–163

Carai MAM, Colombo G, Quang LS, Maher TJ, Gessa GL (2006) Resuscitative treatments on 1,4-butanediol mortality in mice. Ann Emerg Med 47(2):184–189. doi:10.1016/j.annemergmed.2005.10.011

Couper FJ, Marinetti LJ (2002) Hydroxybutyrate(GHB)—effects on human performance and behavior. Forensic Sci Rev 14:102–121

Dawidek-Pietryka K, Szczepaniak S, Dudka J, Mazur M (1998) In vitro studies of human alcohol dehydrogenase inhibition in the process of methanol and ethylene glycol oxidation. Arch Toxicol 72(9):604–607

Dempsey DA, Wiegand TJ, Havel C, Benowitz NL, Haller C (2009) Butanediol (BD) conversion to gamma-hydroxybutyrate, (GHB), is markedly reduced by fomepizole (4-MP), an alcohol dehydrogenase blocker. Clin Toxicol 47(7):113

Duer WC, Byers KL, Martin JV (2001) Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. J Anal Toxicol 25(7):576–582

Eriksson CJP (1983) Human-blood acetaldehyde concentration during ethanol oxidation (update 1982). Pharmacol Biochem Behav 18:141–150

Fung HL, Tsou PS, Bulitta JB, Tran DC, Page NA, Soda D, Fung SM (2008) Pharmacokinetics of 1,4-butanediol in rats: bioactivation to gamma-hydroxybutyric acid, interaction with ethanol, and oral bioavailability. AAPS J 10(1):56–69. doi:10.1208/s12248-007-9006-3

Kintz P, Villain M, Pelissier AL, Cirimele V, Leonetti G (2005) Unusually high concentrations in a fatal GHB case. J Anal Toxicol 29(6):582–585

Lad PJ, Leffert HL (1983) rat-liver alcohol-dehydrogenase.1. purification and characterization. Anal Biochem 133(2):350–361

Lenz D, Rothschild MA, Kroner L (2008) Intoxications due to ingestion of gamma-butyrolactone: organ distribution of gamma-hydroxybutyric acid and gamma-butyrolactone. Ther Drug Monit 30(6):755–761

Lora-Tamayo C, Tena T, Rodriguez A, Sancho JR, Molina E (2003) Intoxication due to 1,4-butanediol. Forensic SciInt 133(3):256–259

Megarbane B, Fompeydie D, Garnier R, Baud FJ (2002) Treatment of a 1,4-butanediol poisoning with fomepizole. J Toxicol Clin Toxicol 40(1):77–80

Poldrugo F, Snead OC (1984) 1,4 butanediol, gamma-hydroxybutyric acid and ethanol—relationships and interactions. Neuropharmacology 23(1):109–113

Poldrugo F, Snead OC (1986) 1,4 butanediol and ethanol compete for degradation in rat-brain and liver invitro. Alcohol 3 (6):367–370

Poldrugo F, Barker S, Basa M, Mallardi F, Snead OC (1985) Ethanol potentiates the toxic effects of 1,4-butanediol. Alcoholism 9 (6):493–497

Quang LS, DeSai MC, Shannon MW, Woolf AD, Maher TJ (2004) 4-methylpyrazole decreases 1,4-butanediol toxicity by blocking its in vivo biotransformation to gamma-hydroxybutyric acid. In: Ali SF, Nabeshima T, Yanagita T (eds) Current status of drug dependence/abuse studies: cellular and molecular mechanisms of drugs of abuse and neurotoxicity, vol 1025. Annals of the New York Academy of Sciences. New York Acad Sciences, New York, pp 528–537. doi:10.1196/annals.1316.065

Reynier M (1969) Pyrazole inhibition and kinetic studies of ethanol and retinol oxidation catalyzed by rat liver alcohol dehydrogenase. Acta Chem Scand 23(4):1119–1129

Shen ML, Lipsky JJ, Naylor S (2000) Role of disulfiram in the in vitro inhibition of rat liver mitochondrial aldehyde dehydrogenase. Biochem Pharmacol 60(7):947–953

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150(1):76–85

Snead OC, Gibson KM (2005) Drug therapy—gamma-hydroxybutyric acid. N Engl J Med 352(26):2721–2732

Theorell H, Yonetani T (1969) On effects of some heterocyclic compounds on enzymic activity of liver alcohol dehydrogenase. Acta Chem Scand 23(1):255–260

Tsukamoto S, Muto T, Nagoya T, Shimamura M, Saito M, Tainaka H (1989) Determinations of ethanol, acetaldehyde and acetate in blood and urine during alcohol oxidation in man. Alcohol Alcohol 24(2):101–108

Zorzano A, Herrera E (1989) Decreased in vivo rate of ethanol-metabolism in the suckling rat. Alcoholism 13(4):527–532

Zvosec DL, Smith SW, McCutcheon JR, Spillane J, Hall BJ, Peacock EA (2001) Adverse events, including death, associated with the use of 1,4-butanediol. N Engl J Med 344(2):87–94