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Butanediol conversion to gamma-hydroxybutyrate markedly reduced by the alcohol dehydrogenase blocker fomepizole

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Key words: 1,4-butanediol, fomepizole, alcohol dehydrogenase, gamma-hydroxybutyrate

Abstract

1,4-Butanediol (BDO) – used as solvent, and abused for its euphoric effects - is converted to gamma-hydroxybutyrate (GHB) by the enzyme alcohol dehydrogenase (ADH). This double-blind, placebo-controlled crossover study with six healthy volunteers is the first to date investigating the role of the ADH inhibitor fomepizole (4MP) in moderating this conversion in humans. Participants received on two different days either intravenous placebo or 15 mg/kg 4MP, followed by oral administration of 25 mg/kg BDO. Pretreatment with 4MP resulted in significantly higher BDO maximal plasma concentration ($p=0.001$) and AUC ($p=0.028$), confirming that ADH is the primary pathway for the conversion of BDO to GHB in humans. With 4MP, the mean arterial pressure was significantly lower at 105 minutes compared to baseline ($p=0.003$), indicating that blood pressure lowering, observed not with a temporal relationship to 4MP administration but after the maximum BDO concentration was reached, may be an intrinsic effect of BDO.

Introduction

Gamma-hydroxybutyrate (GHB) is a therapeutic drug marketed for the treatment of narcolepsy and cataplexy in the form of sodium oxybate as Xyrem® [1-3]. Due to its euphoria-producing effects GHB is also used recreationally [4,5] and has been implicated in drug facilitated sexual assault [6,7]. 1,4-Butanediol (BDO), a readily available industrial chemical found in solvents, is converted to GHB *in vivo* and produces acute as well as chronic and withdrawal effects similar to GHB [8,9]. Since regulatory restriction of GHB was enacted in the United States in 2000, the illicit use of GHB precursors or analogues such as BDO has increased, with several reported cases of BDO abuse, in some cases with fatal outcome [10-13].

Data from previous studies show that BDO is rapidly and extensively converted to GHB after administration, with measurable GHB concentrations within 5 minutes after oral administration [14]. Reported mean maximum drug concentration (Cmax) after administration of 25 mg/kg BDO is 3.8 mg/L, with time of maximum concentration (Tmax) of 26 minutes and elimination half-life (T½) of 39 minutes [14]. The metabolism of BDO is thought to be the same as that of ethanol, with a two-step conversion of BDO to GHB via the hepatic enzyme alcohol dehydrogenase (ADH) to gamma-hydroxybutyraldehyde and then by aldehyde dehydrogenase to GHB [15,16] (Figure 1).

When ethanol is co-ingested with BDO, as is frequently the case in the setting of recreational drug use, this pathway may be competively blocked [17,18], while BDO itself has been shown to potentiate the effects of ethanol in rats [16]. Ethanol competitively inhibited the conversion of BDO to GHB with an apparent K_i of 6.5×10^{-3} M in rat brain and 2.7×10^{-3} M in rat liver [16]. Fomepizole (also known as 4-methylpyrazole, 4MP) is a potent competitive inhibitor of ADH

[19] that is used to treat both methanol and ethylene glycol poisoning [20]. Pretreatment with 4MP in mice increased the Toxic Dose-50 (TD_{50}) of BDO 10-fold for the righting reflex and 30-fold for the rotarod test, two established assessments demonstrating neurologic deficits produced by pharmacological treatments [21], indicating decreased toxicity presumably due to inhibition of the conversion of BDO to GHB.

To date, no study has examined the role of 4MP in moderating the conversion of BDO to GHB in humans. The aim of the present study was to characterize the pharmacokinetics of orally administrated BDO after pharmacological inhibition of ADH by 4MP. We hypothesized that 4MP would block the conversion of BDO to GHB, resulting in persistently high BDO concentrations and low GHB concentrations after pre-treatment with 4MP. This would support the hypothesis that ADH is responsible for the conversion of BDO to GHB. Secondary outcomes included assessing the clinical effects of BDO, as the observations in the presence of persistently high BDO concentrations could help gain a better understanding of the effects of BDO, separately from the effects of its metabolite, GHB.

Results

The mean age of participants was 25.5 years (standard deviation (SD) 4.8), and self-reported races were four White, one Black, and one Asian. The mean body mass index (BMI) was 24.1 kg/m^2 (SD 2.4). By self-report, three volunteers (50%) were cigarette smokers. Mean self-reported alcohol intake was 65 g/week (SD 54.9). The mean administered doses of 4MP and BDO were 1017 mg (SD 155, range 842-1235) and 1695 mg (SD 258, range 1403-2058), respectively.

Pharmacokinetics:

The pharmacokinetics of BDO and GHB for both conditions are summarized in Table 1 and Table 2, respectively.

BDO was rapidly metabolized to GHB in the placebo arm, with three participants having no detectable BDO at any time post dosing (Table 1, Figure 2).

In the 4MP arm, BDO was measurable in all participants up to 360 minutes (after this time point not detectable/below limit of quantification (LOQ) in four and very close to LOQ in two cases), while GHB was not detectable above 5 µg/mL in either arm after 180 minutes (Figures 2 and 3). The last quantifiable GHB concentration (2.5 µg/mL) was measured at 180 minutes in the placebo and at 240 minutes in the 4MP arm. After these time points all values were not detectable/below LOQ.

In the 4MP arm, a BDO mean Cmax of 29.8 µg/mL was reached with a median Tmax of 30 minutes, compared to a mean Cmax of 3.6 µg/mL ($p=0.001$) and Tmax of 15 minutes ($p=0.18$) in the placebo arm (Table 1). A significant difference between the two arms was also seen regarding the area under the BDO plasma concentration-time curve until last measurable concentration (AUC last) ($p=0.028$). The mean T½ of BDO was 162.3 minutes with 4MP pretreatment, while its calculation was not possible in the placebo arm due to values below (n=3) or fluctuating values very close to LOQ (n=3), thus not allowing a confident estimation (Table 1, Figure 2). Consequently, calculation of the extrapolated AUC to infinity (AUC inf) for which the T½ is needed and the oral clearance (Cl/F) which is based on the AUC inf was also not possible for these cases.

Conversely, GHB levels were low in the 4MP arm, and high in the placebo arm, with mean Cmax of 10.9 µg/mL and 50.4 µg/mL, respectively (p=0.001) (Table 2). The T½ of GHB was significantly longer after 4MP (85 minutes) compared to after placebo (35 minutes) (p = 0.008). Further significant differences between the two arms were seen regarding the GHB AUC last (p=0.028) and the AUC inf (p=0.003) (Table 2).

Clinical effects:

The mean heart rate (HR) and mean arterial pressure (MAP) over time during four hours after BDO administration are shown in Figure 4.

There were no significant differences regarding the HR and the oxygen saturation between the two arms or compared to the baseline. Higher MAP values were seen in the placebo compared to the 4MP arm at various time points (Figure 4); however, after adjusting for multiple comparisons using the Bonferroni correction those differences were not significant. Compared to the baseline and after performing the Bonferroni correction for multiple comparisons, significantly lower MAP values were seen in the 4MP arm at 105 minutes (p=0.003), while no significant differences to baseline were seen in the placebo arm.

There were no significant differences in subjective responses between the 4MP and placebo arm or compared to baseline after adjusting for multiple comparisons using the Bonferroni correction. The graphs of the mean scores for all visual analog scale (VAS) questionnaires over time in both treatment arms can be found as Figure S1.

Figure 5 shows the mean VAS score differences to baseline at 30, 60 and 90 minutes after BDO administration in both treatment arms. There were no significant differences regarding the mean VAS score differences to baseline between the 4MP and placebo arm.

Discussion

We present novel data in humans showing that pretreatment with 4MP prior to BDO resulted in significant higher BDO and lower GHB plasma concentrations and AUC, supporting the idea that ADH is the major pathway for the conversion of BDO to GHB. We did observe some production of GHB after BDO in the presence of 4MP. It is unknown if 4MP incompletely blocks the metabolism of BDO to GHB via ADH, or if there are alternative pathways for this conversion. Furthermore, 4MP pretreatment significantly increased the T½ of GHB compared to placebo, which might indicate inhibition of the GHB dehydrogenase (one of the enzymes primary involved in GHB metabolism [22,23]) by 4MP. Subjective effects of BDO were not significantly affected by 4MP treatment, but blood pressure was significantly lower after 4MP compared to placebo. The latter observation could indicate an intrinsic effect of BDO or an effect of 4MP itself.

Reported recreational BDO doses are in the range of 1-14 g in nonfatal and 5.4-20 g (88 to 300 mg/kg) in fatal cases [11]. Reported blood BDO concentrations are 82 µg/mL in one non-fatal intoxication (dose not reported) [12] and 220 µg/mL in another fatal case after ingestion of 5.4-10.8 g (95 – 189 mg/kg) [11]. The BDO dose and blood concentrations in these cases far exceed those observed in our study.

In a previous study on the pharmacokinetics of BDO using the same oral dose as in the present study [14], BDO levels were similar to the present study, showing rapid and extensive conversion to GHB. Also similar to the placebo arm in the present study, participants in the study of Thai et al. reported feeling less alert, decreased concentration and more lightheaded. These findings are in line with known GHB effects from previous studies in which similar GHB concentrations were reached [24,25] and when 25 mg/kg GHB was administered [26], thus supporting the idea that in normal conditions (i.e. no co-administration of other substances) the main effects of BDO are mediated through the action of its metabolite GHB [8].

4MP is a potent competitive inhibitor of ADH and is used clinically in the management of ethylene glycol [27] and methanol poisoning [28]. It is dosed intravenously over 30 minutes every 12 hours, with adjustments for hemodialysis [29]. Potential side effects of 4MP include burning at the infusion site, headache, nausea, dizziness, agitation, eosinophilia, and seizures, although it is unknown whether some of those effects are due to 4MP treatment or the patient's poisoning [20, 27-31]. Inhibition of the BDO metabolism by 4MP has been shown *in vitro* [15,32] and in animal models [15,21,33]. So far as we know, the possible utility of 4MP in prevention of GHB toxicity from BDO in humans has been investigated in only one case report [34]. The patient presented with seizures and coma following ingestion of approximately 3 g BDO; 10 mg/kg 4MP were administered every 12 hours (total of three doses). The BDO plasma concentrations at presentation and at three and eight hours after presentation were 24 µg/mL, 20 µg/mL and undetectable, respectively (delay between ingestion and presentation unknown). The corresponding GHB plasma concentrations were 222 µg/mL, 310 µg/mL, and undetectable, respectively. The patient regained normal consciousness three hours after 4MP administration. Considering the very rapid conversion of BDO to GHB, it is unknown whether 4MP has actually

influenced the recovery course in this case [26]. However, 4MP treatment might provide a potential clinical benefit as antidote in cases of BDO intoxication for patients with slower conversion of BDO to GHB, e.g. in cases of ethanol co-ingestion or due to ADH variant haplotypes associated with slower metabolism [14].

Because of its rapid metabolism to GHB, the physiological actions of BDO itself are currently unknown. Potential alcohol-like effects have been postulated in a previous study [17] based on the potentiation of some effects when BDO was co-administrated with ethanol, but this might have resulted by the potentiation of ethanol's effects due to competition for the same metabolic enzymes [16]. In our study, the significant difference regarding lower blood pressure in the 4MP arm could represent intrinsic effects of BDO. However, we cannot exclude the possibility that the pre-treatment with 4MP and not BDO itself caused the observed effects. Although hypotension is not reported among the common adverse effects of 4MP [20], it has (rarely) occurred according to the product label [29] and there is also one case report available documenting severe hypotension and bradycardia immediately after intravenous fomepizole infusion [35]. The significant lower MAP values in our study were not documented with a close temporal relationship to 4MP administration but after BDO reached its Cmax in the majority of the participants, suggesting that BDO and not 4MP was responsible for these changes.

Limitations of our study include the small sample size, which reduced power to detect differences in subjective responses and did not allow subgroup analysis as sex and racial comparisons. Moreover, a relative small BDO dose was administered, thus not allowing for a generalization of our findings in cases of BDO recreational use where usually higher doses are consumed. Furthermore, no genetic analysis of ADH gene variants with reduced activity was performed. Finally, 4MP was administered before BDO, which would not be the case in a real

life clinical scenario with 4MP use as an antidote. Despite these limitations, our study unequivocally demonstrates that 4MP markedly inhibits the conversion of BDO to GHB in humans.

In summary, pretreatment with intravenous 15 mg/kg 4MP in healthy individuals significantly inhibited the metabolism of BDO to GHB, resulting in significant higher Cmax and AUC of BDO and lower Cmax and AUC of GHB. These findings indicate that ADH is a major pathway for the conversion of BDO to GHB in humans; further investigation of the utility of 4MP as an antidote in cases of BDO intoxication to prevent GHB toxicity is warranted.

Methods

Six consented, healthy volunteers (three males, three females) between the ages of 18 and 45 years participated in a double-blinded, randomized, placebo-controlled, two-arm, crossover study, in which participants received either intravenous placebo (one arm) or 15 mg/kg 4MP (other arm; recommended loading dose for ethylene glycol/methanol poisoning [20]) followed by oral administration of 25 mg/kg BDO (a relative low dose considering reported recreational doses [11]). The washout period between treatments was a minimum of two days.

To avoid first time exposure to a potentially addictive drug, individuals who were drug-naive, (defined as not having ingested GHB, BDO, or related analogues on at least one occasion in the past), were excluded. Also excluded were individuals with a history of illicit drug abuse (other than GHB or cannabis) as assessed by questionnaires or positive urine toxicology test including the following drugs of abuse: amphetamine/methamphetamine/MDMA, barbiturates,

benzodiazepines, cocaine, opiates, methadone, and ethanol. Exclusion criteria also included: significant medical history (e.g. cardiac disease, hypertension, psychiatric condition), conditions requiring regular medication use (with the exception of oral contraceptives), obesity (i.e. BMI ≥30), pregnancy or breastfeeding, alcohol abuse, history of discomfort or difficulties with blood draws. Written informed consent was obtained before enrollment and the study was approved by the Committee on Human Research at the University of California, San Francisco.

Eligibility was determined at a screening visit, including questionnaires, a simple physical examination (vital signs, weight and height), EKG, baseline blood chemistries, and urine testing (for toxicology and pregnancy if applicable). Eligible participants were then scheduled for two separate inpatient study treatment visits. Participants were asked to abstain from certain substances prior to the study day: alcohol and recreational drugs (three days); over-the-counter medications (three days) and dietary supplements (one week); caffeinated beverages and any food (both from midnight the prior evening); any prescription medications that could be safely stopped (one week).

Participants were admitted to the General Clinic Research Center of the Zuckerberg San Francisco General Hospital the morning of the study day at 7am and a single intravenous catheter was placed upon arrival. Doses of 4MP (Antizol®)/placebo were prepared by the hospital Pharmacy, representing a total dose based on 15 mg/kg and diluted in 100 ml normal saline (or just saline for placebo). The drug was administered intravenously at 8am over 30 minutes and was completed 30 minutes prior to BDO dosing (choice of latency time based on the reported Tmax of 30 minutes to two hours following an oral 4MP dose [36]). Doses of BDO were prepared by the hospital Pharmacy, representing a total dose based on 25 mg/kg in liquid

form, and administered orally in water or juice 30 minutes after the end of the 4MP/placebo infusion.

Blood specimens were collected at 0, 5, 15, 30, 45, 60, 90 minutes and 2, 3, 4, 5, 6, 12, 24, and 36 hours after BDO dosing. Plasma samples were frozen and stored for analysis of BDO and GHB concentrations. Plasma was analyzed by gas chromatography – mass spectrometry (GC-MS) for BDO and GHB (LOQ 2 and 2.5 µg/mL, respectively) as described previously [14]; the original method was slightly modified (sonication during derivatization) to improve reproducibility, also resulting in different LOQs. Vital signs were continuously monitored. The MAP was calculated from the systolic and diastolic blood pressure (SP and DP, respectively) using the formula: MAP = DP + 1/3 (SP – DP). Subjective drug effects (i.e. light headed, nausea, shaky/jittery, palpitations, sweaty, flushed, headache, alert, concentration, calm/relaxed, happy/upbeat, irritable/tense) were assessed by VAS questionnaires at 0, 30, 60, 90 minutes and 2, 4, 6 hours. Participants were discharged in the evening after the closeout of the 36 hours.

Plasma concentration-versus-time data for BDO and GHB were evaluated by non-compartmental analysis using WinNonlin (version 6.3; Pharsight Corporation, Mountain View, CA). The T½ was calculated from the slope of the linear portion of the log plasma concentration versus time curve. The Tmax and Cmax were obtained from the individual plasma concentration data. The area under the plasma concentration-time curve was calculated using the trapezoidal rule and was extrapolated from the AUC last to AUC inf. The Cl/F was estimated by dividing the oral dose by the AUC inf.

Numerical data are presented as mean and SD if normally distributed and as median and range if not normally distributed. Baseline of vital signs (HR, MAP and oxygen saturation) was defined as the average of measurements taken at time 60 and 75 minutes before BDO dosing (i.e. before

4MP administration), baseline of VAS scores as the first VAS questionnaire assessment at time 0 (time of BDO administration). For analysis of differences in the VAS scores compared to baseline, the difference for each time point equals the VAS score at this time point minus the VAS baseline score. For graphic representation and descriptive results, concentrations documented as below LOQ/not detected were replaced by 0 and were left blank if no sample was available. Hemodynamic variables were tested by pair-wise comparisons using the paired *t* test. Pharmacokinetic data and VAS scores were tested using the paired *t* test for normally distributed variables, and the Wilcoxon Signed Rank Test for nonparametric results. Due to the 11 and six pairwise comparisons in the hemodynamic variables and the VAS scores, respectively, Bonferroni correction was applied. A p value < 0.05 was considered statistically significant. Data analysis was performed using SPSS statistical software (IBM SPSS Statistics 23.0).

Study Highlights:

- *What is the current knowledge on the topic?*

  1,4-butanediol (BDO) is a readily available industrial solvent. Due to its rapid and extensive metabolism to gamma-hydroxybutyrate (GHB) BDO is abused for its euphoric effects.

- *What question did this study address?*

  This study investigated the potential role of the ADH inhibitor fomepizole (4MP) in moderating the conversion of BDO to GHB in humans.

- *What does this study add to our knowledge?*

Pretreatment with 4MP resulted in significantly higher BDO and lower GHB plasma concentration and area the curve (AUC), thus confirming that ADH is the primary pathway for the conversion. Furthermore, blood pressure was significantly lower after 4MP compared to placebo, which could indicate an intrinsic effect of BDO.

- *How might this change clinical pharmacology or translational science?*
  Based on our findings, further investigation of the utility of 4MP as an antidote in cases of BDO intoxication to prevent GHB toxicity might be warranted.

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References

1. Borgen, L.A., Cook, H.N., Hornfeldt, C.S., Fuller, D.E. Sodium oxybate (GHB) for treatment of cataplexy. *Pharmacotherapy* 22(6), 798-9, discussion 799 (2002)
2. Fuller, D.E., Hornfeldt, C.S., Kelloway, J.S., Stahl, P.J., Anderson, T.F. The Xyrem risk management program. *Drug Saf.* 27(5), 293-306 (2004)
3. Product Information: Xyrem® oral solution, sodium oxybate oral solution. Jazz Pharmaceuticals (per FDA), Palo Alto, CA (2012)
4. Liakoni, E., Walther, F., Nickel, C.H., Liechti, M.E. Presentations to an urban emergency department in Switzerland due to acute γ-hydroxybutyrate toxicity. *Scand. J. Trauma Resusc. Emerg. Med*. 24(1), 107 (2016)
5. Miró, Ò., et al. Intoxication by gamma hydroxybutyrate and related analogues: Clinical characteristics and comparison between pure intoxication and that combined with other substances of abuse. *Toxicol. Lett.* 277, 84-91 (2017)
6. ElSohly, M.A., Salamone, S.J. Prevalence of drugs used in cases of alleged sexual assault. *J. Anal. Toxicol.* 23(3), 141-6 (1999)
7. Gonzalez, A., Nutt, D.J. Gamma hydroxy butyrate abuse and dependency. *J. Psychopharmacol.* 19(2), 195-204 (2005)
8. Irwin, R.D. NTP summary report on the metabolism, disposition, and toxicity of 1,4-butanediol (CAS No. 110-63-4). *Toxic. Rep. Ser.* 54, 1-28, A1-8, B1-5 (1996)
9. Palmer, R.B. Gamma-butyrolactone and 1,4-butanediol: abused analogues of gamma-hydroxybutyrate. *Toxicol. Rev*. 23(1), 21-31 (2004)

10. Shannon, M., Quang, L.S. Gamma-hydroxybutyrate, gamma-butyrolactone, and 1,4-butanediol: a case report and review of the literature. *Pediatr. Emerg. Care* 16(6), 435-40 (2000)

11. Zvosec, D.L., Smith, S.W., McCutcheon, J.R., Spillane, J., Hall, B.J., Peacock, E.A. Adverse events, including death, associated with the use of 1,4-butanediol. *N. Engl. J. Med*. 344(2), 87-94 (2001)

12. Lora-Tamayo, C., Tena, T., Rodríguez, A., Sancho, J.R., Molina, E. Intoxication due to 1,4-butanediol. *Forensic Sci. Int.* 133(3), 256-9 (2003)

13. Theron, L., Jansen, K., Skinner, A. New Zealand's first fatality linked to use of 1,4-butanediol (1,4-B, Fantasy): no evidence of coingestion or comorbidity. *N. Z. Med J.* 116(1184), U650 (2003)

14. Thai, D., Dyer, J.E., Jacob, P., Haller, C.A. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration to healthy volunteers. *Clin. Pharmacol. Ther*. 81(2), 178-84 (2007)

15. Bessman, S.P., McCabe, E.R. 3rd. 1,4-Butanediol--a substrate for rat liver and horse liver alcohol dehydrogenases. *Biochem. Pharmacol.* 21(8), 1135-42 (1972)

16. Poldrugo, F., Snead, O.C. 3rd. 1,4-Butanediol and ethanol compete for degradation in rat brain and liver in vitro. *Alcohol* 3(6), 367-70 (1986)

17. Poldrugo, F., Snead, O.C. 3rd. 1,4 Butanediol, gamma-hydroxybutyric acid and ethanol: relationships and interactions. *Neuropharmacology* 23(1), 109-113 (1984)

18. Schneidereit, T., Burkhart, K., Donovan, J.W. Butanediol toxicity delayed by preingestion of ethanol. *Int. J. Med. Toxicol.* 3, 1 (2000)

19. Blomstrand, R., Theorell, H. Inhibitory effect on ethanol oxidation in man after administration of 4-methylpyrazole. *Life Sci. II* 9(11):631-40 (1970)

20. Brent, J. Fomepizole for ethylene glycol and methanol poisoning. *N. Engl. J. Med*. 360(21), 2216-23 (2009)

21. Quang, L.S., Shannon, M.W., Woolf, A.D., Desai, M.C., Maher, T.J. Pretreatment of CD-1 mice with 4-methylpyrazole blocks toxicity from the gamma-hydroxybutyrate precursor, 1,4-butanediol. *Life Sci.* 71(7), 771-8 (2002)

22. Kaufman, E.E., Nelson, T., Goochee, C., Sokoloff, L. Purification and characterization of an NADP+-linked alcohol oxido-reductase which catalyzes the interconversion of gamma-hydroxybutyrate and succinic semialdehyde. *J. Neurochem.* 32(3):699-712 (1979)

23. Nelson, T., Kaufman, E.E. Developmental time courses in the brain and kidney of two enzymes that oxidize gamma-hydroxybutyrate. *Dev. Neurosci.* 16(5-6):352-8 (1994)

24. Brenneisen, R., et al. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J. Anal. Toxicol.* 28(8), 625-30 (2004)

25. Ferrara, S.D., et al. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br. J. Clin. Pharmacol*. 34(3), 231-5 (1992)

26. Busardò, F.P., Jones, A.W. GHB pharmacology and toxicology: acute intoxication, concentrations in blood and urine in forensic cases and treatment of the withdrawal syndrome. *Curr. Neuropharmacol.* 13(1), 47-70 (2015)

27. Brent, J., et al. Fomepizole for the treatment of ethylene glycol poisoning. Methylpyrazole for Toxic Alcohols Study Group. *N. Engl. J. Med*. 340(11):832-8 (1999)

28. Brent, J., McMartin, K., Phillips, S., Aaron, C., Kulig, K. Methylpyrazole for Toxic Alcohols Study Group. Fomepizole for the treatment of methanol poisoning. *N. Engl. J. Med*. 344(6):424-9 (2001)

29. Product Information: Antizol®, fomepizole. Orphan Medical, Inc., Minnetonka, MN, (2000)

30. Mégarbane, B., et al. Treatment of acute methanol poisoning with fomepizole. *Intensive Care Med.* 27(8):1370-8 (2001)

31. Jacobsen, D., Sebastian, C.S., Blomstrand, R., McMartin, K.E. 4-Methylpyrazole: a controlled study of safety in healthy human subjects after single, ascending doses. *Alcohol Clin. Exp. Res.* 12(4):516-22 (1988)

32. Lenz, D., et al. Inhibition of 1,4-butanediol metabolism in human liver in vitro. *Naunyn Schmiedebergs Arch. Pharmacol*. 383(6), 647-54 (2011)

33. Carai, M.A., Colombo, G., Quang, L.S., Maher, T.J., Gessa, G.L. Resuscitative treatments on 1,4-butanediol mortality in mice. *Ann. Emerg. Med*. 47(2), 184-9 (2006)

34. Mégarbane, B., Fompeydie, D., Garnier, R., Baud, F.J. Treatment of a 1,4-butanediol poisoning with fomepizole. *J. Toxicol. Clin. Toxicol.* 40(1), 77-80 (2002)

35. Lepik, K.J., et al. Bradycardia and hypotension associated with fomepizole infusion during hemodialysis. *Clin. Toxicol. (Phila.)* 46(6), 570-3. (2008)

36. Jacobsen, D., Barron, S.K., Sebastian, C.S., Blomstrand, R., McMartin, K.E. Non-linear kinetics of 4-methylpyrazole in healthy human subjects. *Eur. J. Clin. Pharmacol*. 37(6):599-604 (1989)

Figure Legends

Figure 1. Chemical structures and metabolic pathway for 1,4-butanediol to gamma-hydroxybutyric acid (generally referred to as gamma-hydroxybutyrate (GHB))

Figure 2. Mean (± standard error) plasma concentrations of 1,4-butanediol (BDO) over time in both conditions (inset: semi-logarithmic plot of the data; 4MP: fomepizole; n=6, except at 5 and 720 minutes in the placebo arm and at 45 minutes in the 4MP arm (n=5 due to missing data))

Figure 3. Mean (± standard error) plasma concentrations of gamma-hydroxybutyrate (GHB) over time in both conditions (inset: semi-logarithmic plot of the data; 4MP: fomepizole; n=6, except at 5 and 720 minutes in the placebo arm and at 45 minutes in the 4MP arm (n=5 due to missing data))

Figure 4. Mean (± standard error) a. heart rate (HR) and b. mean arterial pressure (MAP) over time in the fomepizole (4MP) (n=6, except at 30 and 45 minutes for MAP (n=5)) and the placebo arm (n=6, except at 105 minutes (n=5))

Figure 5. Subjective mood and symptoms: Mean change (± standard error) in scores compared to baseline at a. 30 minutes, b. 60 minutes and c. 90 minutes (n=6)

Supplementary Material: Figure S1

Table 1. Pharmacokinetics of 1,4-butanediol (BDO) after pretreatment with placebo or 4MP (mean (SD) or median (range)) (n=6, unless indicated otherwise)

	Placebo	**4MP**	***p***
Tmax (min)	15 (5-30)[§]	30 (30-180)	0.18
Cmax (µg/mL)	3.6 (4.3)	29.8 (11.5)	**0.001**
T½ (min)	n/a	162.3 (47.8)	n/a
AUC last (min*µg/mL)	179.4 (0-9412.0)	6110.0 (4732.8-12392.2)	**0.028**
AUC inf (min*µg/mL)	n/a	8192.1 (5413.7-13493.6)	n/a
Cl/F (mL/min)	n/a	205.3 (66.3)	n/a

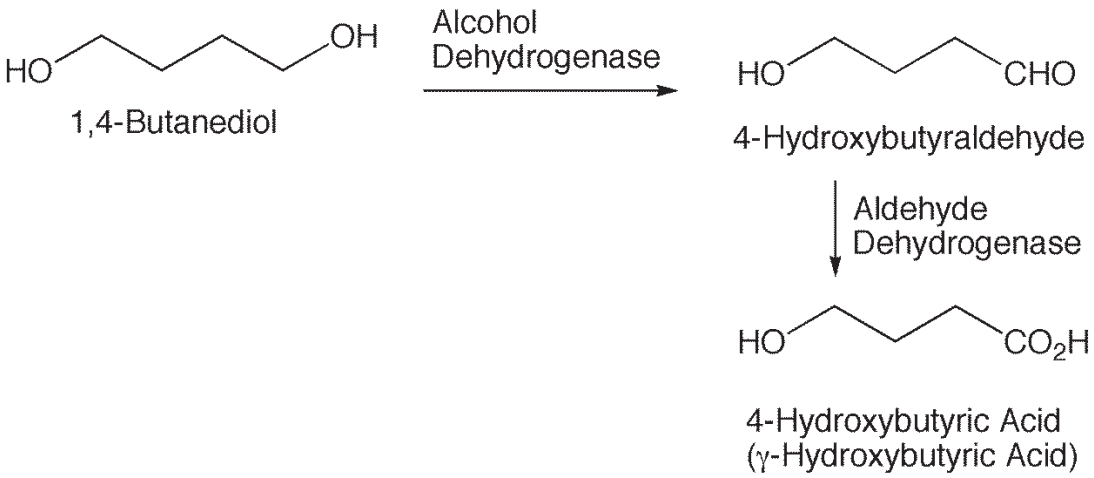
[§]n=3 (for the other three all concentrations below LOQ/not detected)

4MP: fomepizole; Tmax: time of maximum concentration; Cmax: maximum concentration; T½: elimination half-life; AUC: area under the plasma concentration-time curve (last: until last measured concentration; inf: extrapolation to infinity); Cl/F: oral clearance

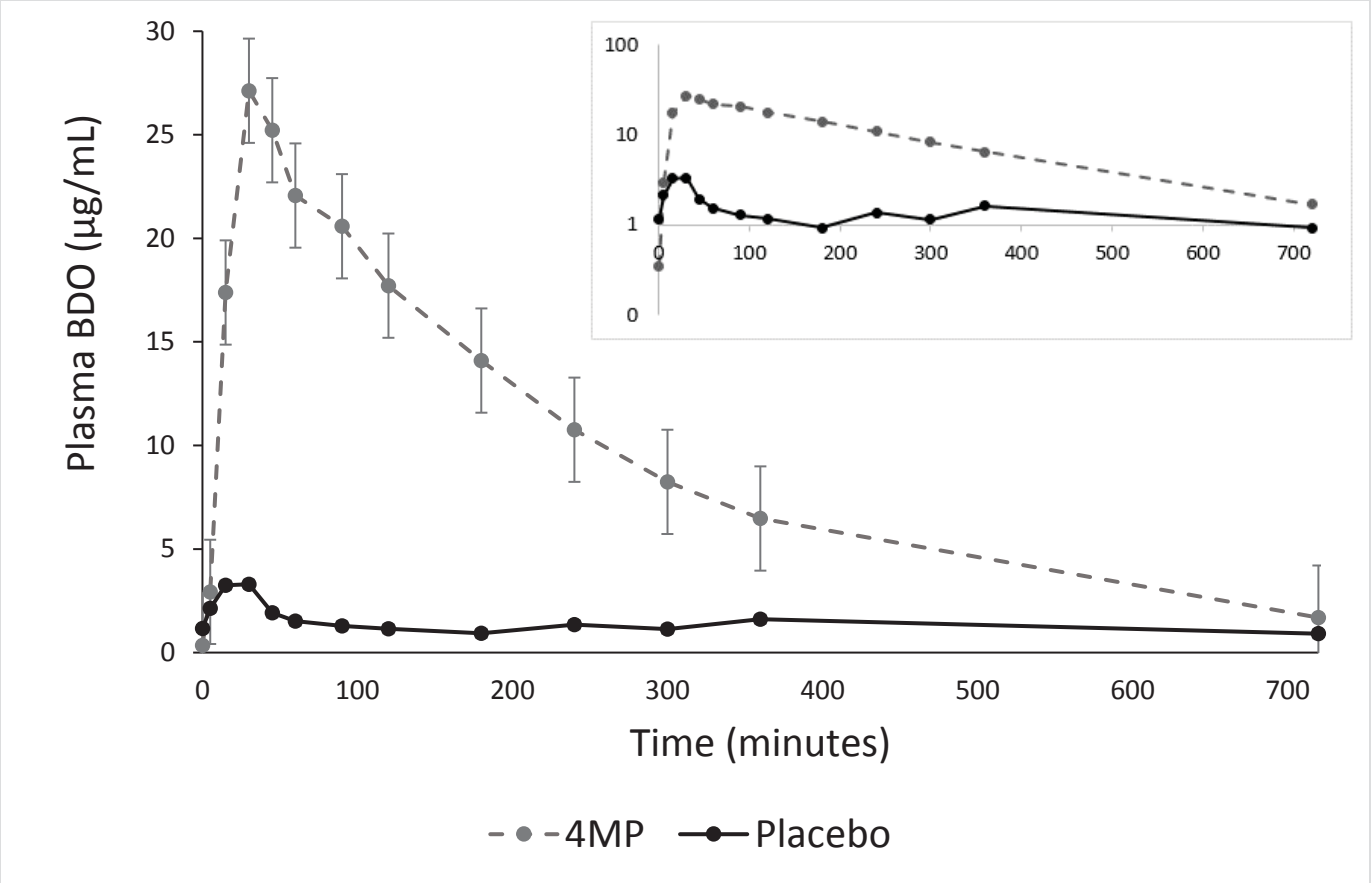
Table 2. Pharmacokinetics of gamma-hydroxybutyrate (GHB) after pretreatment with placebo and 4MP (mean (SD) or median (range)) (n=6, unless indicated otherwise)

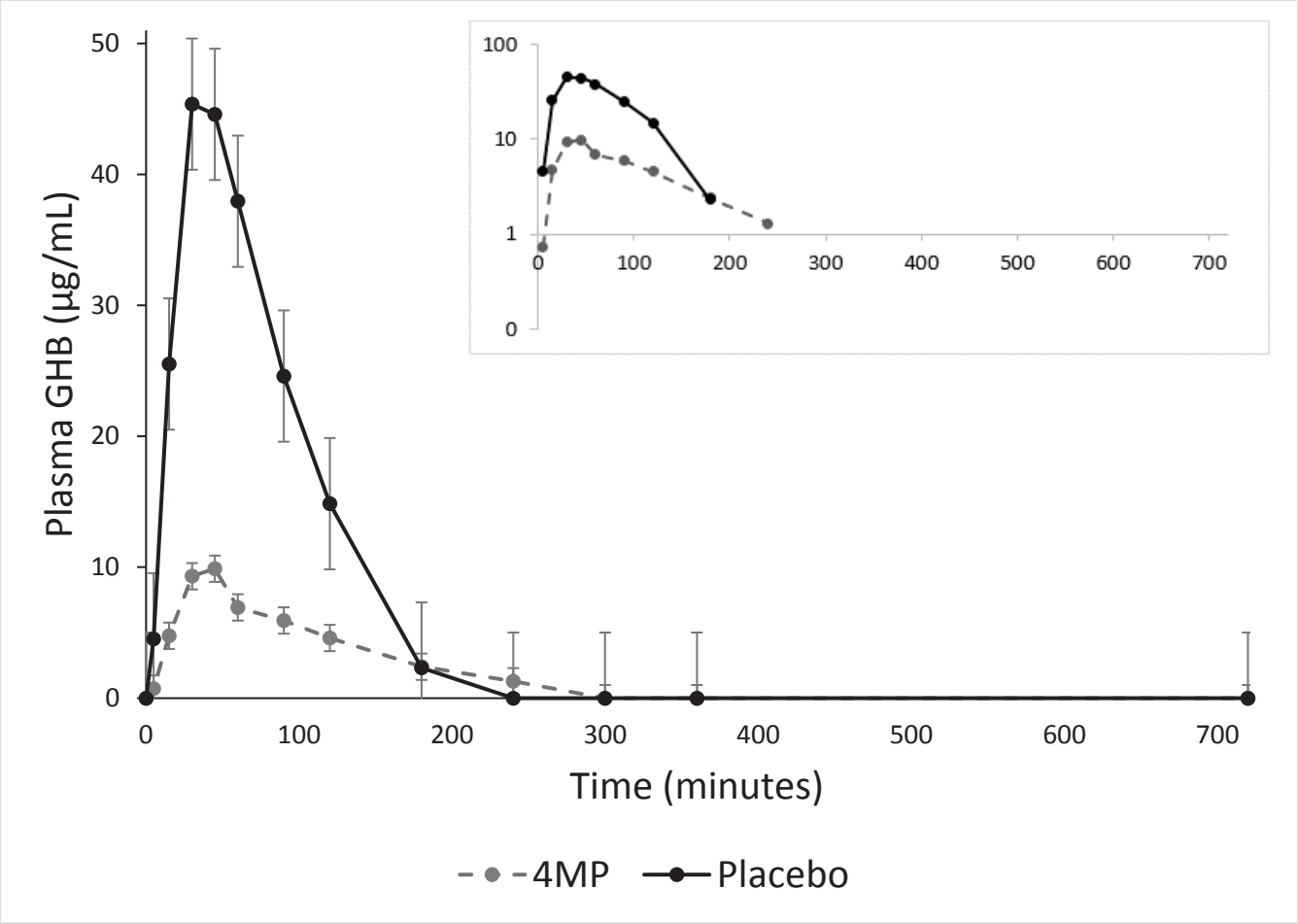
	Placebo	**4MP**	***p***
Tmax (min)	40.0 (12.2)	47.5 (24.0)	0.518
Cmax (µg/mL)	50.4 (15.1)	10.9 (4.3)	**0.001**
T½ (min)	35 (5.0)	85 (25.3)[§]	**0.008**
AUC last (min*µg/mL)	3611.8 (2839.5-6001.1)	917.4 (871.1-1466.1)	**0.028**
AUC inf (min*µg/mL)	4320.3 (1196.2)	1485.5 (366.6)[§]	**0.003**

[§] n=5 (T½, and consecutive AUC inf, due to very low concentrations not calculable in one case)
4MP: fomepizole; Tmax: time of maximum concentration; Cmax: maximum concentration; T½: elimination half-life; AUC: area under the plasma concentration-time curve (last: until last measured concentration; inf: extrapolation to infinity); Cl/F: oral clearance

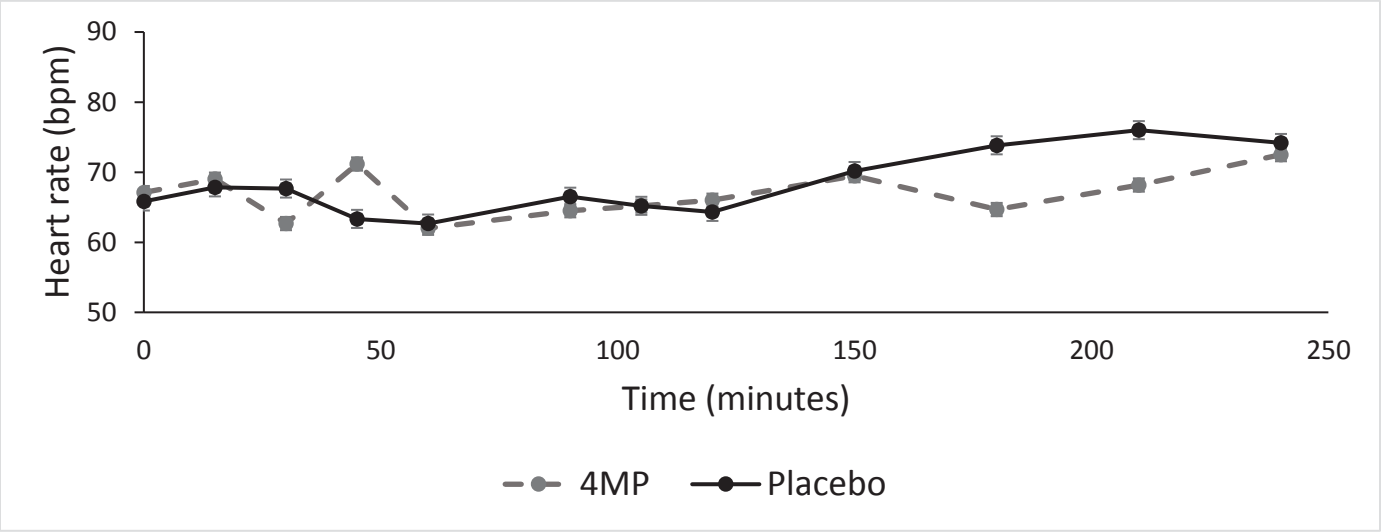

HO
OH
1,4-Butanediol
Alcohol
Dehydrogenase
HO
CHO
4-Hydroxybutyraldehyde
Aldehyde
Dehydrogenase
HO
CO2H
4-Hydroxybutyric Acid
(γ-Hydroxybutyric Acid)

GHB

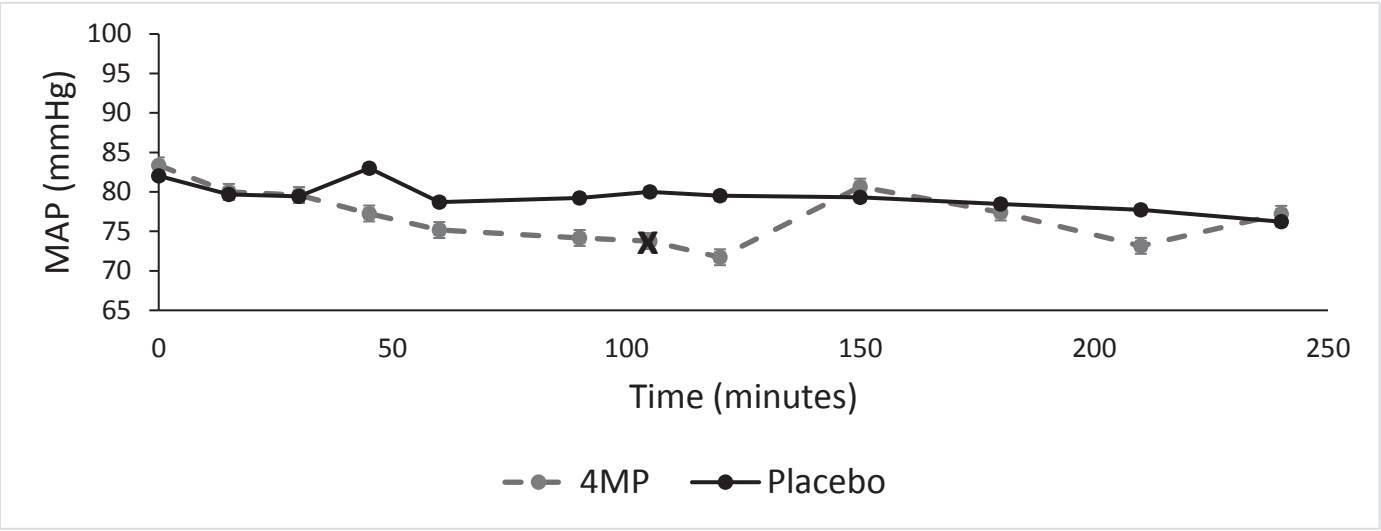

Plasma BDO (µg/mL)
30
25
20
15
10
5
0
0
100
200
300
400
500
600
700
Time (minutes)
4MP
Placebo
100
10
1
0


Plasma GHB (µg/mL)
50
40
30
20
10
0
0
100
200
300
400
500
600
700
Time (minutes)
4MP
Placebo
100
10
1
0

a.

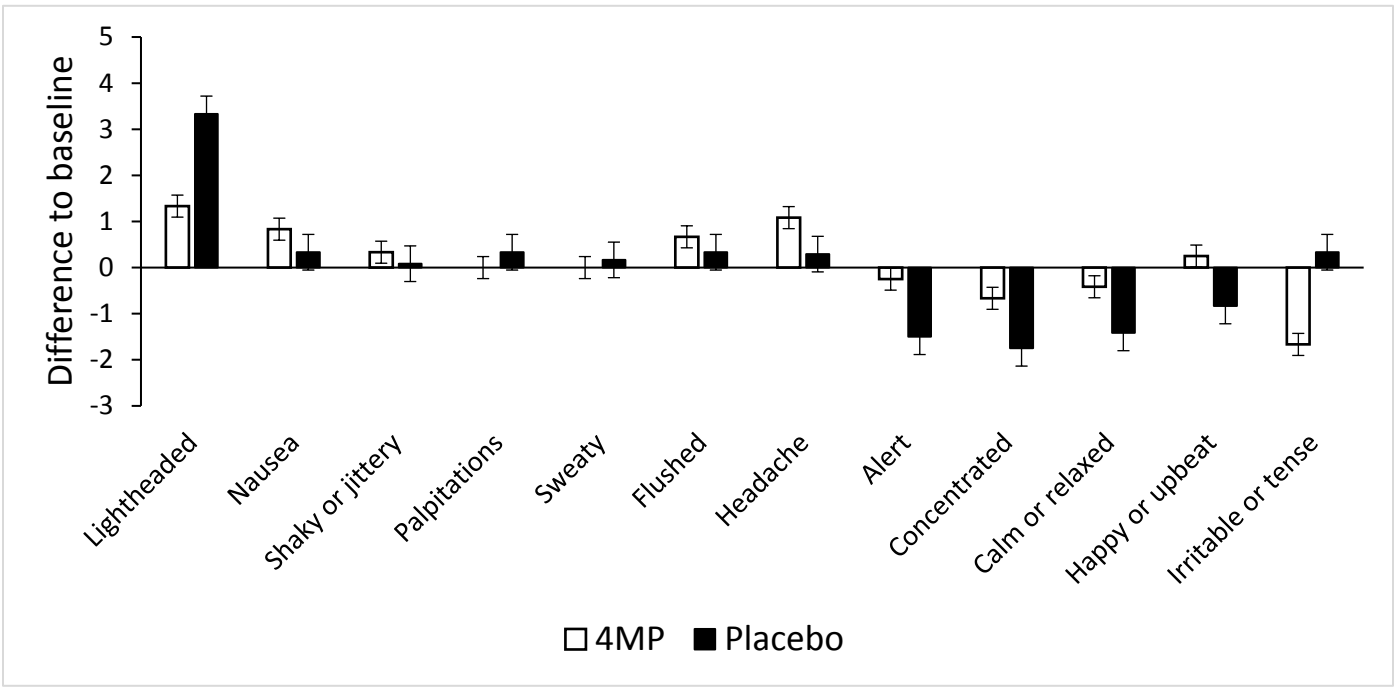


b.

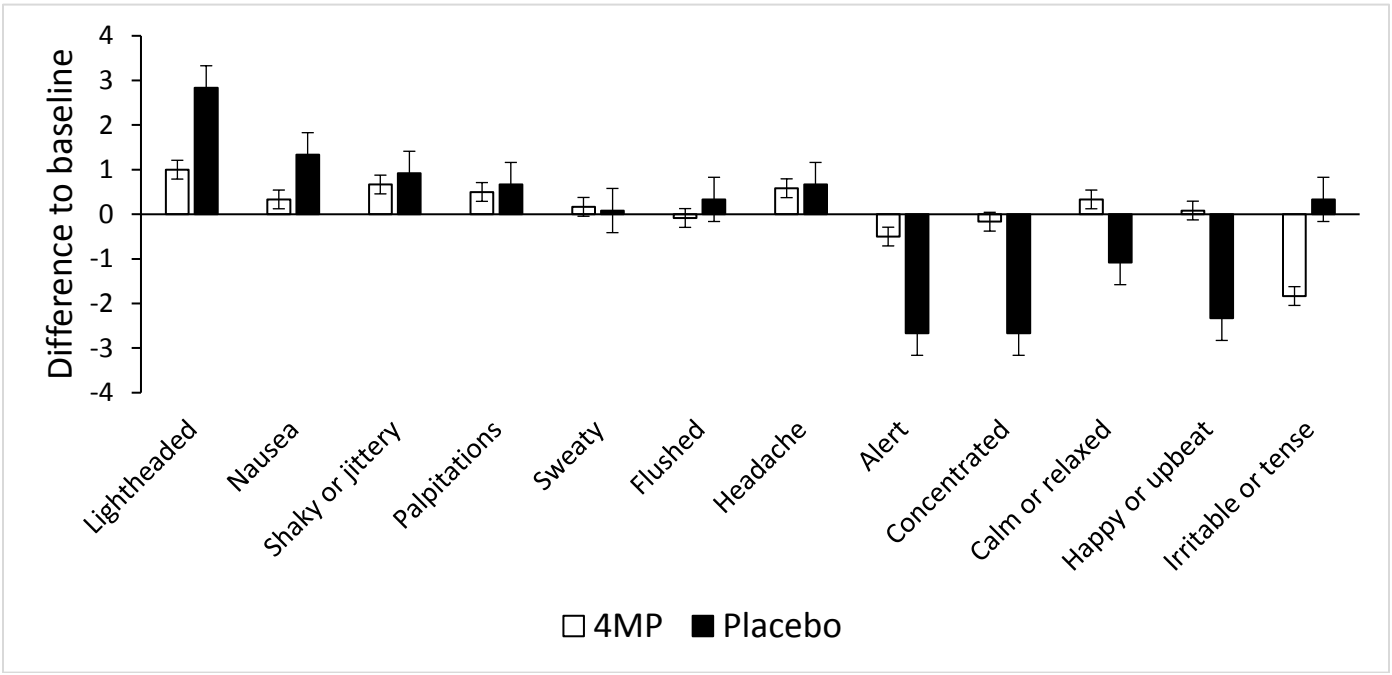


(time points marked with x indicate significant difference (p = 0.003) compared to baseline after adjusting for multiple comparisons using the Bonferroni correction)

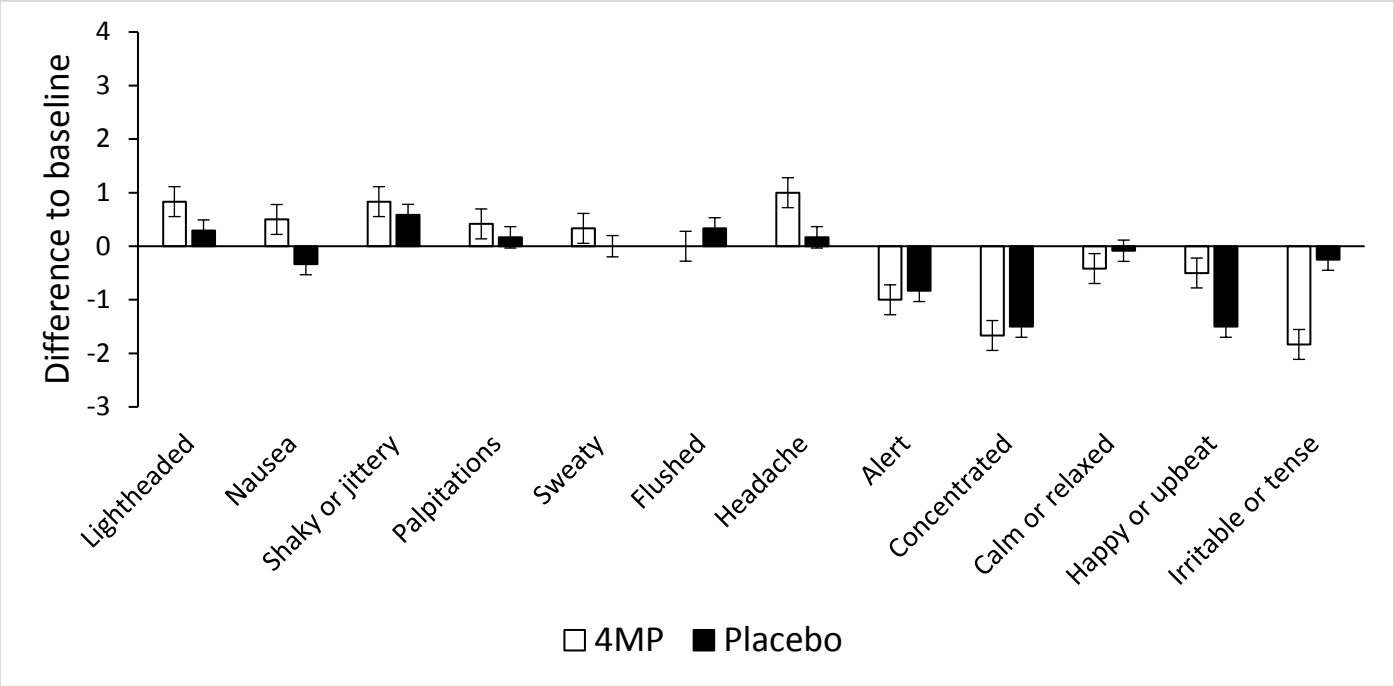
a.


Difference to baseline
5
4
3
2
1
0
-1
-2
-3
Lightheaded
Nausea
Shaky or jittery
Palpitations
Sweaty
Flushed
Headache
Alert
Concentrated
Calm or relaxed
Happy or upbeat
Irritable or tense
4MP
Placebo

b.


Difference to baseline
4
3
2
1
0
-1
-2
-3
-4
Lightheaded
Nausea
Shaky or jittery
Palpitations
Sweaty
Flushed
Headache
Alert
Concentrated
Calm or relaxed
Happy or upbeat
Irritable or tense
4MP
Placebo

c.


Difference to baseline
4
3
2
1
0
-1
-2
-3
Lightheaded
Nausea
Shaky or jittery
Palpitations
Sweaty
Flushed
Headache
Alert
Concentrated
Calm or relaxed
Happy or upbeat
Irritable or tense
4MP
Placebo