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Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers

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Summary. Gamma-hydroxybutyric acid (GHB) is effective in treatment of the alcohol and opiate withdrawal syndromes. Its absorption and disposition kinetics have been studied in 8 healthy male volunteers following oral administration of single doses of 12.5, 25 and 50 $mg\,kg^{-1}$.

The AUC increased disproportionately with the dose and so the apparent oral clearance decreased significantly as the dose was increased, whereas the terminal half-life and mean residence time increased. The peak plasma concentrations normalised to the lowest dose fell significantly with increasing doses, whilst the corresponding peak times increased.

These findings suggest that both the oral absorption and the elimination of GHB are capacity-limited processes. GHB did not bind to significant extent to plasma proteins over the therapeutic concentration range.

The pharmacokinetic parameters in healthy volunteers were not significantly different from those previously observed in alcohol-dependent patients with compensated alcoholic liver disease.

Key words: Gamma-hydroxybutyric acid; pharmacokinetics, dose-proportionality

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Gamma-hydroxybutyric acid (GHB) is an endogenous constituent of the mammalian brain, where it is synthesized from gamma-aminobutyric acid (GABA) [1, 2]. Evidence has accumulated that GHB is not just a metabolite of GABA and that it plays a role as a central neurotransmitter or neuromodulator (see 3 for review). GHB was formerly used as an intravenous anaesthetic agent [4] and in the treatment of narcolepsy [5]. It has recently been reintroduced into therapeutics for the treatment of alcohol dependence [6]. Given daily in oral doses of 50 to 100 $mg\,kg^{-1}$, GHB rapidly suppresses alcohol withdrawal symptoms, and reduces alcohol comsumption and craving without causing any serious side-effects [6, 7]. A pharmacokinetic study has recently been conducted in alcohol-dependent patients [8]. Consistent with the rapid onset and short duration of the effect of GHB, the study showed that GHB absorption and elimination were fast processes. Virtually no unchanged drug could be recovered in the urine, in accordance with previous animal studies, which indicated that GHB was almost exclusively cleared by hepatic biotransformation [3]. Preliminary indications have also been obtained of non-linear kinetic behaviour.

The present study had three main purposes:

1. To determine the pharmacokinetic parameters of GHB in healthy volunteers, since no information was available from normal subjects. It is known that long-term alcohol abuse may enhance or decrease hepatic drug metabolism as a consequence of enzyme induction or hepatocyte dysfunction [9]. Thus, pharmacokinetic information obtained in alcohol abusers may not be relevant to normal subjects. Pharmacokinetic information in non-alcoholics is necessary because of recent clinical observations that GHB is not only useful in alcohol dependence, but it is also effective in preventing and suppressing opiate withdrawal symptomatology [10].
2. To examine the dose-proportionality of GHB after administration of ascending therapeutic oral doses.
3. To assess the plasma protein binding of GHB and its possible concentration dependence.

Subjects and methods

Subjects

Eight, healthy, nonsmoking male volunteers, aged 22 to 26 y, and weighing 66 to 85 kg (mean 79.2 kg, SD 7.5 kg), gave informed written consent to participation in the study, which was approved by the University of Padova Medical School Ethics Committee. All participants were diagnosed as healthy by means of a thorough clinical examination, including medical history, physical examination, complete blood count and laboratory tests, indicating normal function of the kidney (serum creatinine and blood urea nitrogen) and liver (direct and total serum bilirubin, serum protein and albumin, alanine and aspartate aminotransferases, gamma-glutamyltransferase, prothrombin time). The subjects were instructed to avoid any other drugs, including alcohol, for 2 weeks before the study and during the entire period of investigation.

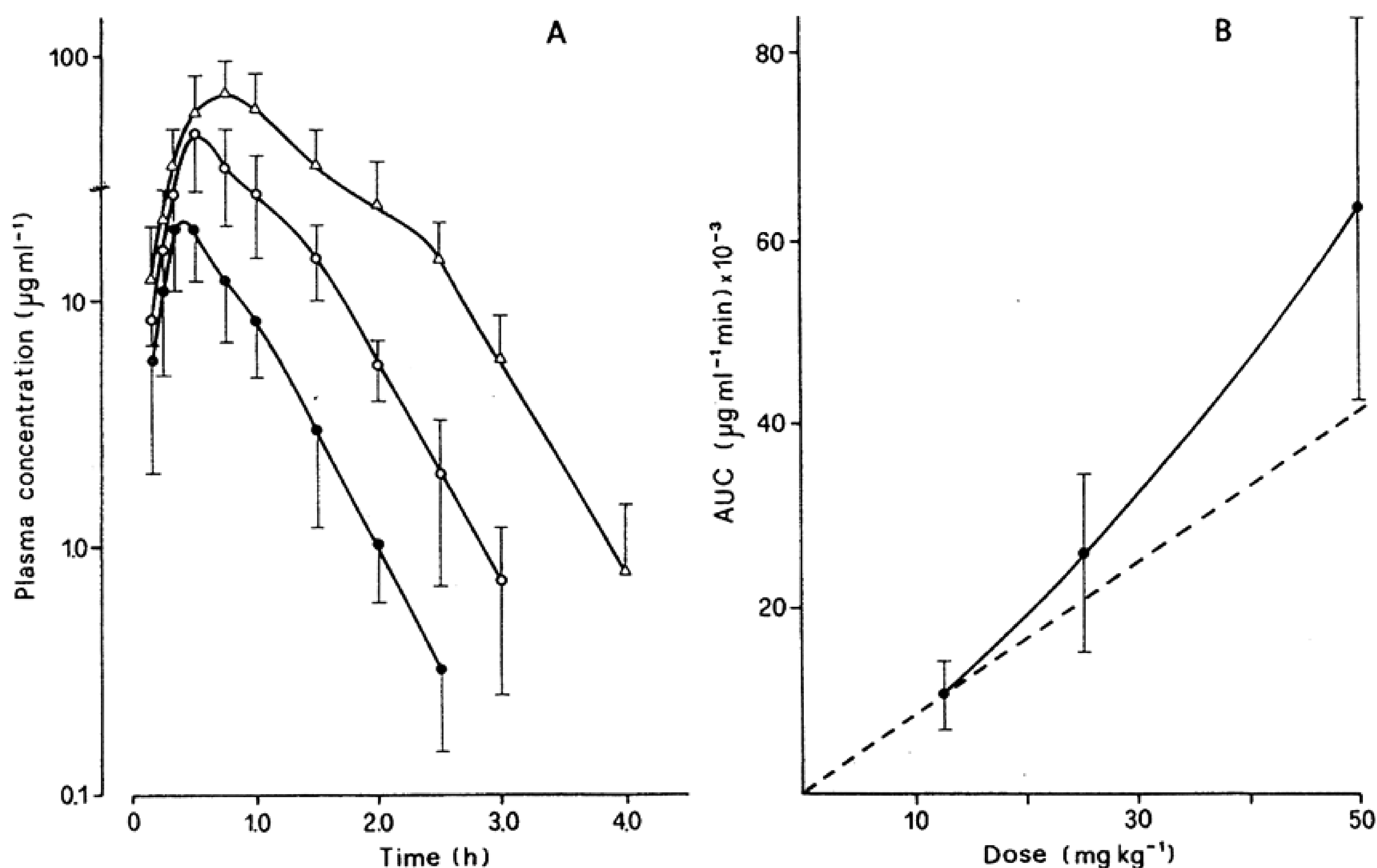

Fig. 1. A. Semilogarithmic plots of mean (SD) plasma concentrations of GHB following oral administration of 12.5 (●), 25 (○) and 50 (△) $mg\,kg^{-1}$.
B shows the relationship between AUC and dose of GHB. The *dotted line* is the relationship anticipated from the lowest AUC-dose data pair on the basis of linear kinetics

Study design

At 08.00 h, after an overnight fast, GHB dissolved in a black cherry syrup (CT, Sanremo, Italy) was given orally to the 8 volunteers in doses of 12.5, 25 and 50 $mg\,kg^{-1}$. The different doses were given in a random order, with a washout period of 3 days between each dose. The appropriate volumes of syrup were diluted to 100 ml with water and the cup containing GHB was rinsed with a further 50 ml water, so that the total fluid intake was 150 ml for all doses. The volunteers remained sitting for the first 2 h after dosing, after which, they were allowed a further drink of water and were permitted to walk in the ward. A light standard meal was provided after 4 h.

Blood samples were collected through an indwelling catheter into heparinised plastic tubes at 0 (predose), 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4 and 6 h after dosing. All subjects were closely monitored for possible adverse effects during the entire course of the study.

Analytical methods

Plasma GHB was determined by a gas chromatographic/mass spectrometric method [8, 11]. The assay was linear over the clinically relevant concentration range (2–200 $\mu g\,ml^{-1}$) with a correlation coefficient of 0.999. The detection limit was 0.2 $\mu g\,ml^{-1}$. The intra- and inter-assay coefficients of variation ($n = 5$) at 5 and 100 $\mu g\,ml^{-1}$ were below 5%.

The plasma protein binding of GHB at 37° was determined in duplicate by equilibrium dialysis, using a Dianorm® equilibrium dialyser (Diachema AG, Switzerland) equipped with 1 ml cells and semipermeable membranes with a molecular weight cut-off of 5.000 D. Preliminary experiments established that equilibrium was attained within 1 h and that there was no difference in binding between plasma and serum. The possible concentration dependence of GHB protein binding was evaluated in the plasma of a single volunteer at predialysis concentrations of 3, 10, 20, 100, 200, 300 $\mu g\,ml^{-1}$. As no concentration-dependent binding was observed, the plasma protein binding in each subject was determined at a single GHB concentration. GHB was added to 0.9 ml of a predose plasma sample to produce a concentration of 25 $\mu g\,ml^{-1}$, and the pH was adjusted to 7.4 with 0.3 M phosphoric acid. The plasma was dialysed against an equal volume of 0.13 $mol \cdot l^{-1}$ phosphate buffer pH 7.4 for 1 h and the GHB concentration was then determined in aliquots taken from both chambers. The fraction of unbound drug (f_u) was calculated as the ratio of the concentration in buffer to that in plasma. Allowance was not made for volume shift (< 10%), since the error introduced by ignoring it was negligible at the observed degree of binding [12].

Pharmacokinetic and statistical analyses

Pharmacokinetic parameters were estimated by standard non-compartmental methods. The peak plasma GHB concentration (C_{max}) and the time of its occurrence (t_{max}) were the observed values. Terminal half-life ($t_{1/2z}$) was obtained by log-linear regression analysis of the terminal phase of the concentration-time curves. The areas under the plasma drug concentration-time curves (AUC) and under the first moment of the plasma drug concentration-time curves (AUMC) were calculated by the linear trapezoidal rule up to the last determined concentration, and were extrapolated to infinity by standard methods [13]. The extrapolated portion was always less than 10% of the total area. Mean residence time (MRT) was calculated as AUMC/AUC and apparent oral clearance (CL_o) as dose/AUC.

Pharmacokinetic parameters are expressed as means (SD), with the exception of t_{max}, for which the median value (range) is reported. Statistical comparisons were made by two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS® (1988) Release 6.03. SAS Institute, Cary, NC, USA). Wilcoxon's signed rank test was used as the non-parametric test of differences in t_{max}. A $P < 0.05$ was considered statistically significant.

Results

The time course of the plasma GHB levels after administration of 12.5, 25 and 50 $mg\,kg^{-1}$ is shown in Fig. 1 A. After each dose, the semilogarithmic plot of concentration-time data exhibited a biphasic decay phase: an initial rapid decline followed by a convex concentration-time profile, which became increasingly prominent as the dose was raised. Such a decay pattern is typical of drugs with a pronounced distributive phase and non-linear elimination kinetics [13]. Increasing the dose caused a disproportion-

Table 1. Mean (SD) pharmacokinetic parameters of GHB after administration of different doses to 8 healthy volunteers

Parameter	Dose ($mg\,kg^{-1}$)		
	12.5	25	50
AUC ($\mu g\,ml^{-1}\,min$)	905 (443)	1271 (560)[a]**	1565 (548)[a]**
CL_o ($ml\,min^{-1}\,kg^{-1}$)	14 (6)	9 (4)*	7 (3)**
MRT (min)	45 (10)	53 (9)**	70 (12)**
$t_{1/2z}$ (min)	20 (2)	22 (3)	23 (3)*
c_{max} ($\mu g\,ml^{-1}$)	23 (9)	23 (11)[a]	20 (7)[a]*
t_{max} (min)	25 (20–30)[b]	30 (20–45)[b]*	45 (30–60)[b]**
f_u	0.99 (0.03)[c]		

[a] Normalized to 12.5 $mg\,kg^{-1}$; [b] Median value (range); [c] Determined at a predialysis concentration of 25 $\mu g\,ml^{-1}$ (see Methods); * $P < 0.05$ and ** $P < 0.01$ relative to values in the 12.5 $mg\,kg^{-1}$ dose group

ate increase in AUC (Fig. 1B), thereby confirming the [no]nlinearity of GHB elimination kinetics. Accordingly, there was a significant and progressive increase in dose-normalised AUC as the dose was raised (Table 1). As a consequence, large variations were recorded in CL_o and MRT. However, $t_{1/2z}$ changed to a much more limited ex[te]nt. Increasing the dose did produce a significant increase [in] t_{max} with a concomitant decrease in dose-normalised C_{max} (Table 1). This suggests that the absorption of GHB is capacity-limited in the therapeutic dose range. It can also be appreciated that the free fraction of GHB in plasma approached 1, indicating no significant protein binding of the drug.

Statistical comparison of the present results with data previously obtained in alcohol-dependent-subjects [8] revealed that, at equal doses, the pharmacokinetic parameters did not differ significantly between the two groups ($P > 0.05$ for all parameters).

After the 12.5 $mg\,kg^{-1}$ dose, three subjects reported slight dizziness, which occurred around t_{max} and lasted about 15 min. After the doses of 25 and 50 $mg\,kg^{-1}$ all volunteers complained of dizziness and/or drowsiness. The symptoms were still mild and subsided completely within 20 to 60 min, with the exception of three subjects, who, after the 50 $mg\,kg^{-1}$ dose, also complained of nausea for 60 to 90 min. The peak concentrations in those subjects (56 to 98 $\mu g\,ml^{-1}$) were similar to those observed in the other subjects.

Discussion

Previous studies have shown that the elimination kinetics of GHB is non-linear in animals [15–18]. The results of the present investigation indicate that GHB elimination kinetics is also non-linear in normal human subjects over the therapeutic dose range. A plasma decay profile quite similar to that observed here was obtained by van der Pol et al. following IV administration of 60 $mg\,kg^{-1}$ GHB (unpublished data reported in Ref. 19). Such a decay pattern was interpreted as reflecting the presence of parallel first-order and capacity-limited alimination pathways [19; see also 13, pp. 282–4]. As GHB is not excreted by the kidneys [8], the most likely explanation for the observed non-linearity is saturation of one or more of its as yet poorly defined metabolic pathways [3]. However, it cannot be excluded that saturable cellular uptake may be responsible for the dose-dependent kinetics of GHB, since active transport of the drug has been documented in the rat [18].

In apparent contrast to the large reduction in CL_o, which was halved upon increasing the dose from 12.5 to 50 $mg\,kg^{-1}$, $t_{1/2z}$ increased by only 15 %. This cannot be ascribed to variation in the apparent volume of distribution, since GHB does not bind to plasma proteins; the apparent volume of distribution of GHB in rats was shown to be invariant with dose [17]. The most likely explanation for this apparent discrepancy is that $t_{1/2z}$ reflects the slope of the terminal portion of the curve, which is essentially independent of the dose, since the drug concentration was no longer saturating.

Oral administration of ascending doses of GHB resulted in an increase in t_{max} and a decrease in normalised c_{max}, suggesting capacity-limited absorption of GHB. The fact that the modification of c_{max} was not as prominent as that of t_{max} may have been due to the concomitant saturation of the elimination process, which made c_{max} values higher than expected from linear elimination kinetics, thereby masking the effect of saturable absorption. A quite similar dose-related absorption pattern has been observed in the rat, where saturable transport across the intestinal mucosa has been demonstrated [17, 18].

The pharmacokinetic parameters of GHB observed here in healthy volunteers proved to be very similar to those previously obtained from a group of alcohol-dependent patients with compensated alcoholic liver disease [8]. Thus, as long as hepatic function remains in a compensated state, alcohol abuse does not appear to affect GHB elimination. In spite of similar peak plasma concentrations, the frequency of concentration-related side-effects was higher in healthy volunteers than in alcohol-dependent patients (only 20% of the latter subjects complained of dizziness or drownsiness; 8). However, tolerance to these symptoms readily develops [6, 7].

On the basis of the present results, it may be concluded that the same dosing regimen can be used for alcoholic and non-alcoholic subjects. However, a greater fractionation of the daily dose of GHB appears preferable for the latter subjects, in order to avoid concentration-related adverse effects during the early phase of therapy.

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References

1. Roth RH, Giarman NJ (1969) Conversion in vivo of gamma-aminobutyric to gamma-hydroxybutyric acid in the rat. Biochem Pharmacol 18: 247–250
2. Snead OC, Morley BJ (1981) Ontogeny of gamma-hydroxybutyric acid. Regional concentration in developing rat, monkey and human brain. Brain Res 227: 579–589
3. Vayer P, Mandel P, Maitre M (1987) Gamma-hydroxybutyrate, a possible neurotransmitter. Life Sci 41: 1547–1557
4. Laborit H, Jovany JM, Gerard J, Fabiani F (1960) Sur un substrat metabolique à action centrale inibitrice. Le 4-hydroxybutyrate de Na. Press Med 50: 1867–1869

5. Mamelak M, Scharf MB, Woods M (1986) Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. Sleep 9: 285–289
6. Gallimberti L, Canton G, Gentile N, Ferri M, Cibin M, Ferrara SD, Fadda F, Gessa GL (1989) Gamma-hydroxybutyric acid for treatment of alcohol withdrawal syndrome. Lancet 2: 787–789
7. Gallimberti G, Ferri M, Ferrara SD, Fadda F, Gessa GL (1992) Gamma-hydroxybutyric acid in the treatment of alcohol dependence: a double blind study. Alcohol Clin Exp Res 16: 673–676
8. Ferrara SD, Zotti S, Tedeschi L, Frison G, Castagna F, Gallimberti L, Gessa GL, Palatini P (1992) Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. Br J Clin Pharmacol 34: 231–235
9. Hoyumpa AM, Schenker S (1982) Major drug interactions: effect of liver disease, alcohol and malnutrition. Ann Rev Med 33: 113–149
10. Gallimberti L, Cibin M, Pagnin P, Sabbion R, Pani PP, Pirastu R, Ferrara SD, Gessa GL (1993) Gamma-hydroxybutyric acid for treatment of opiate withdrawal syndrome. Neuropsychopharmacology 9: 77–82
11. Ferrara SD, Tedeschi L, Frison G, Castagna F, Gallimberti L, Giorgetti R, Gessa GL, Palatini P (1993) Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography/mass spectrometry. J Pharmaceut Biomed Anal 11: 483–487
12. Huang J-D (1983) Errors in estimating the unbound fraction of drugs due to the volume shift in equilibrium dialysis. J Pharm Sci 72: 1368–1369
13. Gibaldi M, Perrier R (1982) Pharmacokinetics. Dekker, New York
14. Deleted
15. van der Pol W, van der Kleijn E, Lauw M (1975) Gas chromatographic determination and pharmacokinetics of 4-hydroxybutyrate in dog and mouse. J Pharmacokinet Biopharm 3: 99–113
16. Shumate JS, Snead OC (1979) Plasma and central nervous system kinetics of gamma-hydroxybutyrate. Res Commun Chem Pathol Pharmacol 25: 241–256
17. Lettieri JT, Fung HL (1979) Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. J Pharmacol Exp Ther 208: 7–11
18. Arena C, Fung HL (1980) Absorption of sodium gamma-hydroxybutyrate and its prodrug gamma-butyrolactone: relationship between in vitro transport and in vivo absorption. J Pharm Sci 69: 356–358
19. van Ginneken CAM, van Rossum JM, Fleuren HLJM (1974) Linear and nonlinear kinetics of drug elimination. J Pharmacokinet Biopharm 2: 395–415

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