Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 4, pp. 317–321, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Study of bioequivalence of magnesium and sodium valproates

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Abstract: The *in vivo* bioavailability of magnesium valproate (500 and 1000 mg) enteric-coated tablets has been compared with that of sodium valproate (Depakine®) (500 and 1000 mg) enteric-coated tablets. The two preparations were found to be bioequivalent; magnesium valproate appeared to be a drug without bioavailability problems and with reduced inter-subject variability, compared with that of sodium valproate. A reversed-phase HPLC method for the determination of valproates is described.

Keywords: *Magnesium valproate; sodium valproate; bioequivalence; serum analysis; reversed-phase HPLC.*

Introduction

Valproic acid (VPA) is an antiepileptic drug that has been developed relatively recently. Its therapeutic efficacy in the treatment of epilepsy has been established [1].

VPA is available in pharmaceutical preparations either as the sodium salt (NaVPA) or the magnesium salt (MgVPA). The latter salt is marketed in non-European countries; use of MgVPA is based on a rationale favouring magnesium salts in the treatment of epilepsy [2–5].

A few studies on the comparative bioavailability of a NaVPA formulation and a VPA formulation have been performed and the two preparations were found to be bioequivalent. Enteric coating or controlled release did not affect the bioavailability [6, 7]; however, no estimation of MgVPA bioavailability has been reported.

If active VPA is released sufficiently from the pharmaceutical matrix of the formulation no problems in its bioavailability are to be expected. Nevertheless in the case of a drug intended for chronic use, especially in children, it is of extreme importance to determine its bioequivalence against a widely used reference formulation.

Therefore a comparative *in vivo* study of MgVPA against NaVPA (Depakine®) was undertaken on 10 healthy volunteers. Serum VPA levels were determined by a modification of the HPLC method proposed by Moody and Allan [8].

Experimental

Subjects

Ten healthy male volunteers were studied, aged 24–27 years (mean: 24.9 ± 1.45 years) and of weight 60–80 kg (mean: 69.3 ± 6.75 kg); they were recruited from a population of students after excluding those with any evidence or history of relevant diseases, drug allergy or drug dependence. The subjects were informed about the nature of the compound they were going to receive and gave written consent.

Randomized design

The subjects were given placebo, NaVPA 500 or 1000 mg (calculated as VPA) or MgVPA 500 or 1000 mg (calculated as VPA), in a randomized cross-over design, in a series of five experimental sessions. Each subject received all preparations and served as his own control. The five sessions were carried out at weekly intervals.

Drug administration

The tablets were administered orally in a single dose with 100 ml of water, 2 h after a light standard breakfast. During the study no

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restriction on fluid intake was imposed but food was not allowed.

In no case had subjects been exposed to any drug therapy during the 3 weeks preceding the beginning of the study. They were requested to abstain from taking any neuroactive substance (including alcohol) in the 72 h preceding and in the 24 h following each experimental session.

Biological samples

Under baseline conditions before drug administration, and 1, 2, 3, 4, 6 and 8 h after administration, 10 ml of venous blood was drawn, heparinized, centrifuged and frozen at −20°C.

Apparatus and chromatographic conditions

The apparatus comprised a Perkin–Elmer Model Series 4 chromatograph (Norwalk, PA, USA), a Model Lambda-3 UV adapted as a detector (254 nm) with an 8-μl flow cell and a Model Data Station 3700 or LCI-100 integrator to record and elaborate the analytical data. The injector valve was a Rheodyne 7125 (Berkeley, CA, USA) valve equipped with a 20-μl loop. A 250 × 4 mm i.d. stainless-steel Hibar column (Merck, Darmstadt, FRG) prepacked with 5-μm Lichrosorb RP-18 (Merck) was used. The column was protected with a 25 × 4 mm i.d. guard column dry-packed with 10-μm ODS (Riedel de Haen, Hanover, FRG).

The mobile phase was acetonitrile–phosphate buffer (pH 7.0) (80:20, v/v); the flow rate was 1.8 ml min^{-1}.

Chemicals and reagents

All salts and solvents were HPLC grade. Phosphate buffer (pH 7.0, 0.35 M) was prepared by dissolving 19.04 g of KH_2PO_4 and 62.64 g of $Na_2HPO_4 \cdot 6H_2O$ in 1 l of fresh distilled water. Stock solutions of valproic acid (VPA standard; 2.4 g l^{-1}) and nonanoic acid (internal standard; 100.0 g l^{-1}) were prepared in double-distilled water for VPA and in acetonitrile for nonanoic acid. Appropriate dilutions of the stock solutions were made before use.

The esterifying agent was a solution of 4-bromophenacyl bromide (0.4 g/20 ml) and dicylohexane-18-crown-6 in acetonitrile (10 mg/20 ml).

Sample preparation

In a 1.5-ml conical centrifuge tube, a mixture of 100 μl of serum or VPA standard, 25 μl (0.35 M) of phosphate buffer and 250 μl (0.1 mg l^{-1}) of internal standard were vortexed for 5 s and centrifuged for 5 min; 200 μl of the supernatant was transferred into a 50-mm glass tube containing 50 μl of esterifying agent. The mixture was heated at 70°C for 15 min. After cooling a 20-μl aliquot was injected into the chromatograph.

Standard solutions

The working VPA solution was prepared by diluting the stock solution (1:10) to give a concentration of 0.24 g l^{-1}. To obtain the working internal standard solution 10 μl of the mother solution was diluted to 10 μl with acetonitrile (0.1 mg l^{-1}).

Calibration curve

To obtain the calibration curve, 100 μl of the working VPA solution was diluted to 1 μl with blank serum. Aliquots of this solution were further diluted to obtain a series of solutions with concentrations of 5–60 μg ml^{-1}, which were treated in an identical manner as previously described for the sample preparation. A 20-μl aliquot was injected and the ratio between mean drug peak area and that of the internal standard (y) was plotted against the corresponding concentration (x). Each standard was replicated (n = 5). The linear regression equation was: $y = 0.011x - 0.15$ (r = 0.999).

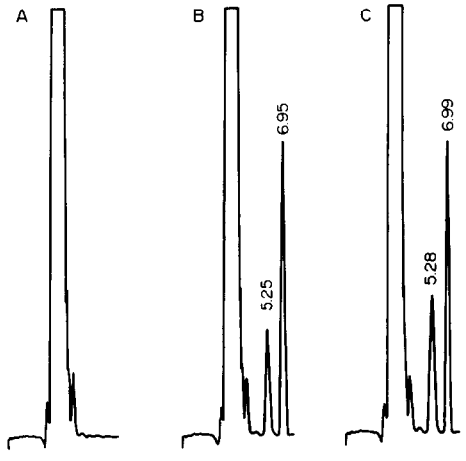

Figure 1
Representative chromatograms of VPA in serum: A, drug-free serum; B, serum obtained 4 h after administration of MgVPA (1000 mg); C, standard of 45 μg ml^{-1}. For chromatographic conditions, see text. VPA: RT = 5.28. Internal standard: RT = 6.99.

Results and Discussion

The proposed method proved to be suitable for the determination of VPA in serum and no interference from serum compounds was observed. Representative chromatograms are shown in Fig. 1.

VPA was identified by comparing its retention time with that of the standard. The limit of detection (LOD) for VPA calculated according to [9] was 1.7 ± 0.4 μg ml^{-1}. The precision of the analysis of VPA in patient samples was evaluated in three pools of six samples each at VPA concentrations of 15, 30 and 45 μg ml^{-1}. Recovery values are given in Table 1. The reproducibility determined by injecting the same solution eight times was good with a RSD of 2.80% at a concentration of 40 μg ml^{-1}.

The serum concentration of the tested drugs is illustrated in Figs 2 and 3 as a function of time.

Table 1
Recovery of VPA from serum

VPA added (μg ml^{-1})	Mean recovery (μg ml^{-1})	±SD
15	13.58	1.43
30	27.85	1.69
45	43.32	2.02

The serum concentration of VPA reached detectable levels after administration, regardless of the preparation and the dose. Peak levels were usually reached after administration of the 1000 mg dose, whereas a plateau was evident 3–7 h after a 500 mg dose. Detectable values for serum concentration were still measured 1 week after administration of the drug (Tables 2 and 3).

The serum concentrations after administration of equivalent doses of NaVPA and

Table 2
Serum concentration at different times after drug administration. The mean for subjects is expressed as μg ml^{-1}, with standard deviation. Statistical comparison between compounds by the Mann–Whitney test for ranked data (z-score)

		Time from administration (h) 1	2	3	4	5	6	8	1 week
NaVPA (500 mg)	χ	4.7	10.6	18.3	22.9	25.8	25.5	23.2	3.0
	SD	2.14	8.85	13.54	11.30	6.43	7.84	6.74	3.46
MgVPA (500 mg)	χ	6.3	14.5	22.5	23.5	23.1	23.0	22.2	3.7
	SD	6.87	10.81	4.51	4.85	4.66	4.62	3.24	3.20
	z	0.038	0.454	0.379	0.038	0.645	0.681	0.227	0.879
	p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NaVPA (1000 mg)	χ	4.6	10.6	21.5	37.8	47.7	45.6	45.2	4.7
	SD	10.52	17.78	19.59	20.18	14.10	13.75	12.40	3.9
MgVPA (1000 mg)	χ	6.8	16.7	23.4	35.2	41.1	41.5	39.4	1.9
	SD	5.93	13.27	17.53	13.78	14.66	14.66	12.35	2.38
	z	2.338	1.987	n.s.	n.s.	n.s.	n.s.	n.s.	2.244
	p	0.019	0.049	n.s.	n.s.	n.s.	n.s.	n.s.	0.025

Table 3
Inter-subject relative standard deviation (%) of VPA serum concentration (μg ml^{-1}) at different times after drug administration

	Time from administration (h) 1	2	3	4	5	6	8	1 week
NaVPA (500 mg)	0.45	0.83	0.73	0.49	0.25	0.31	0.29	1.15
MgVPA (500 mg)	1.09	0.74	0.20	0.20	0.20	0.20	0.14	0.86
NaVPA (1000 mg)	2.28	1.68	0.91	0.53	0.29	0.30	0.27	0.65
MgVPA (1000 mg)	0.87	0.79	0.75	0.39	0.31	0.35	0.31	1.25

Table 4
AUC, C_{max} and t_{max} values calculated after administration of enteric-coated tablets of MgVPA or NaVPA (Depakine)

	MgVPA (500 mg)			MgVPA (1000 mg)			NaVPA (500 mg)			NaVPA (1000 mg)		
Volunteer	AUC* (mg l^{-1} h^{-1})	C_{max} (μg ml^{-1})	t_{max} (h)	AUC* (mg l^{-1} h^{-1})	C_{max} (μg ml^{-1})	t_{max} (h)	AUC* (mg l^{-1} h^{-1})	C_{max} (μg ml^{-1})	t_{max} (h)	AUC* (mg l^{-1} h^{-1})	C_{max} (μg ml^{-1})	t_{max} (h)
1	261.5	20.5	6	449.0	51.5	5	229.0	19.5	3	622.0	55.0	5
2	197.5	20.0	3	362.5	44.0	5	203.5	23.0	5	439.0	51.0	5
3	292.5	30.0	5	580.5	76.0	4	157.5	19.0	5	534.0	50.5	4
4	349.5	33.0	4	558.5	44.5	5	325.5	31.5	5	404.5	43.5	5
5	246.0	29.0	5	377.5	43.5	6	275.0	25.5	3	547.5	69.5	6
6	313.0	25.0	6	284.0	23.5	5	312.5	29.0	5	257.0	38.0	5
7	300.0	26.0	3	397.0	35.5	7	267.0	25.5	4	210.0	28.0	7
8	303.5	27.5	6	625.5	55.5	5	477.0	44.0	5	683.0	59.0	5
9	305.5	25.5	4	911.5	51.5	5	371.5	34.5	5	584.5	65.5	5
10	303.5	24.5	3	286.0	38.0	5	218.0	28.5	7	410.5	39.5	5
Mean + SD	287.3 ± 42.2	26.1 ± 4	4.5 ± 1.3	443.2 ± 121.6	46.4 ± 13.9	5 ± 1.5	283.7 ± 92.9	28.1 ± 7.4	4.7 ± 1.2	469.3 ± 154.0	50.0 ± 12.9	5 ± 1.5
RSD	15%			27%			33%			33%		

NaVPA (500 mg) vs MgVPA (500 mg†): AUC: $z = 0.318$, n.s.; C_{max}: 0.866, n.s.; t_{max} $z = 0.241$, n.s.
NaVPA (1000 mg) vs MgVPA (1000 mg†): AUC: $z = 0.642$, n.s.; C_{max}: 1.173, n.s.; t_{max} $z = 0.423$, n.s.

* Area under the plasma concentration–time curve.
† Wilcoxon's rank test between compounds.

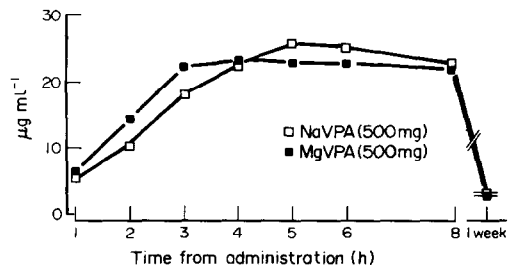

Figure 2
Serum concentration of VPA versus time (500 mg NaVPA and MgVPA).

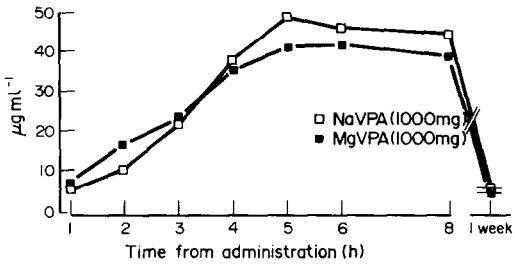

Figure 3
Serum concentration of VPA versus time (1000 mg NaVPA and MgVPA).

MgVPA were substantially superimposable. However, the concentration of valproic acid after administration of MgVPA was significantly higher than that after administration of NaVPA for samples taken 1 and 2 h after administration of the 1000 mg dose. The variability of most of the concentration values was lower after MgVPA than after the equivalent dose of NaVPA (Table 3). The AUC C_{max} and t_{max} after administration of MgVPA were similar to that after NaVPA (Table 4).

MgVPA and NaVPA proved to be substantially equivalent at the doses administered and under the experimental conditions designed for this study; the variability, both in respect of subjects and time, of the drug serum levels was smaller after MgVPA compared with that of the standard compound at doses corresponding to those usually administered daily to patients of average body weight. This characteristic is potentially beneficial in the treatment of epilepsy, where the therapeutic efficacy largely depends on the drug bioavailability at appropriate serum concentrations with a steady-state profile over time and minimal fluctuations below the "therapeutic" levels [10–12].

Acknowledgements — The authors wish to thank SIGMA-TAU Farmaceutici, Roma, Italy, for supplying the compounds tested in the study. This work was supported by National Research Council (CNR) Roma (Italy).

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[Received for review 16 February 1990; revised manuscript received 6 December 1990]