Once-a-Day Controlled-Release Dosage Form of Divalproex Sodium I: Formulation Design and In Vitro/In Vivo Investigations

YIHONG QIU, HOWARD S. CHESKIN, KEVIN R. ENGH, RICHARD P. POSKA

Formulation Center, Global Pharmaceutical Research and Development, Abbott Laboratories, 1401 N. Sheridan Rd, North Chicago, Illinois 60064

Received 15 October 2002; revised 10 December 2002; accepted 18 December 2002

ABSTRACT: Divalproex sodium is a narrow therapeutic index drug that is widely used for the treatment of epilepsy, the manic episodes associated with bipolar disorder, and prophylaxis of migraine headaches. The present investigation was undertaken to design an oral dosage form that would provide once-daily administration with improved therapy and to explore the relationships between in vitro drug release and in vivo absorption. Controlled release hydrophilic matrix formulations of divalproex sodium were designed and evaluated via in vitro and in vivo studies. The release rate of divalproex sodium was modulated by varying different rate-controlling hydrophilic polymers and measured in vitro using a USP apparatus II dissolution method. Formulations with differing release rates were studied in beagle dogs and in healthy subjects. A selected formulation given once-daily was further evaluated against the commercial enteric tablet dosed twice-daily in a multiple dose study, and shown to provide desired nearly constant therapeutic plasma concentrations over the entire 24-h dosing interval. Preliminary linear relationships between in vitro dissolution and in vivo absorption were observed in both the animal model and in humans. However, the relationships were formulation dependent, indicating a need for further studies. © 2003 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 92:1166–1173, 2003

Keywords: divalproex sodium; controlled release; matrix system; in vitro release; in vivo absorption; in vitro—in vivo relationship

INTRODUCTION

Divalproex sodium is a stable coordination compound comprised of sodium valproate and valproic acid in a 1:1 molar relationship and formed during the partial neutralization of valproic acid with 0.5 equivalent of sodium hydroxide. Chemically it is designated as sodium hydrogen bis(2-propylpentanoate) (Figure 1). The anhydrous divalproex sodium exists as crystalline aggregates that appear as waxy white flakes with melting point of approximately 100°C. Divalproex sodium has a relatively high aqueous solubility at neutral pH (e.g., 36 mg/mL at pH 6.2). However, the solubility decreases with pH (e.g., 2 mg/mL at pH 4.7, pKₐ = 4.7). It is stable in the solid state, and dissociates to the valproate ion in the gastrointestinal tract before being absorbed. Mean terminal elimination half-life for valproate monotherapy ranged from 9 to 16 h following oral dosing regimens of 250 to 1000 mg.¹

Divalproex sodium has antiepileptic activity against a variety of types of seizures with minimal sedation, other CNS, and gastrointestinal side effects. It is also indicated for the treatment of the manic episodes associated with bipolar disorder and for prophylaxis of migraine headaches.¹,² It is one of the most widely used bipolar and antiepileptic agents.³ Because divalproex sodium is a

Correspondence to: Yihong Qiu (Telephone: 847-938-5220; Fax: 847-935-1997; E-mail: qiu.yihong@abbott.com)
narrow therapeutic index drug that requires chronic administration, a controlled-release dosage form may provide increased clinical value over conventional formulations as a result of improved patient compliance, a decreased incidence, and/or intensity of the side effects and a more constant or prolonged therapeutic effect.

The objectives of the present study were to design a controlled-release tablet formulation of divalproex sodium intended for once-daily administration and to explore the relationship between in vitro release and in vivo absorption.

EXPERIMENTAL

Materials and Equipment

The following materials and equipment were used in the study: Divalproex sodium (Pharmaceutical Products Division, Abbott Laboratories), Methocel K100LV, CR, Methocel K4MP CR, Methocel K15MP CR, Methocel K100MP CR (Dow Chemical Co.), Keltone HVCR (Kalco, FMC Biopolymers), Depakote® Tablet, 500 mg (Abbott laboratories). Collette high shear mixers (model Gral-10, Gral-75), Wiscosin oven (model batch 3/3/4-5), ValKel tablet hardness analyzer (model VK2000), Carver Press (model C), Compu-Trac™ moisture analyzer (model NA-5), Stokes tablet machine (model B-2), Vanderkamp dissolution tester (model 600), Abbott TDx® Analyzer.

Formulations and Tablet Preparation

During early phase of feasibility studies, both hydrophobic and hydrophilic matrices were tested for providing extended drug release in vitro and in animal models. Experiments continued with only hydrophilic matrices because these systems do not leave behind “ghost” tablets following complete drug release, and also offer significant advantages in processing. Hydrophilic polymers evaluated for preparing matrix tablets include: (a) hydroxypropyl methylcellulose (HPMC), Methocel K100LV, K15M, and K100MP, and (b) sodium alginate, Keltone HVCR.

The tablet formulations evaluated consisted of divalproex sodium, polymer(s), fillers, and magnesium stearate or silicon dioxide. Selected formulations used in in vitro and in vivo studies are given in Table 1. Matrix tablets were produced by wet granulation using a high shear mixer. The polymer was dry mixed with drug and other excipients in the high shear mixer for 5 min. Wet granules were prepared by adding 70 ml/kg of granulation fluid to the polymer powder mixture over a 1–2-min period at high impeller speed. Additional fluid was added as needed. The granules were tray dried at 55°C until moisture content of not more than 1% was obtained as measured by loss on drying (LOD). The dried granules were screened through a 20-mesh sieve and blended with the lubricant in a V-blender for 5 min. One gram tablets were compressed using a hydraulic press or rotary tabletting machines using an oval punch (0.36 x 0.747 in). The prepared matrix tablets were subject to hardness and drug release testing.

In Vitro Release Tests

The in vitro release rates of valproate from matrix tablets were determined using the USP apparatus II, i.e., paddle method. The paddle rotation speed was kept at 100 rpm, and a temperature of 37 ± 0.5°C was maintained. Release testing was carried

Table 1. Composition of Controlled-Release Hydrophilic Matrix Formulations of Divalproex Sodium

<table>
<thead>
<tr>
<th>Formulation</th>
<th>A (w/w%)</th>
<th>B (w/w%)</th>
<th>C (w/w%)</th>
<th>D (w/w%)</th>
<th>E (w/w%)</th>
<th>F (w/w%)</th>
<th>G (w/w%)</th>
<th>H (w/w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Divalproex sodium</td>
<td>53.8%</td>
<td>50–53.8%</td>
<td>50%</td>
<td>53.8%</td>
<td>53.8%</td>
<td>53.8%</td>
<td>53.8%</td>
<td>50%</td>
</tr>
<tr>
<td>Methocel K100LV</td>
<td>18%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Methocel K15M</td>
<td>8%</td>
<td>30%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>35%</td>
<td>20%</td>
<td>40%</td>
</tr>
<tr>
<td>Methocel K100MP</td>
<td>—</td>
<td>—</td>
<td>15%</td>
<td>30%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Keltone HVCR</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fillers/lubricants</td>
<td>20.2%</td>
<td>16.2–20%</td>
<td>35%</td>
<td>16.2%</td>
<td>11.2%</td>
<td>26.2%</td>
<td>16.2%</td>
<td>20%</td>
</tr>
</tbody>
</table>
out in 900 mL of pH 6.8 phosphate buffer. In some cases, 0.1 M HCl was used as the dissolution medium for the first hour. Samples of 1.5 mL were withdrawn at predetermined time intervals up to 24 h, and replaced with an equal volume of the fresh medium. Samples were filtered through a 35-μm filter and assayed by a TDx® fluorescence-polarized immunoassay using an automated TDx® system. Valproate calibrators consisting of six standard concentrations (0, 12.5, 25, 50, 100, and 150 μg/mL) were used to perform an assay specific calibration prior to sample analysis. Dissolution samples were then pipetted into a sample cartridge and loaded into the analyzer along with valproate controls and monoclonal II reagent pack for automated sample analysis. The sensitivity of the assay was 0.70 μg/mL. High accuracy and precision were also confirmed (average recovery = 100.7 ± 2.2%; CV within run = 1.95–2.21%; CV between day = 1.23–1.27%).

In Vivo Studies

Single-Dose Studies in Beagle Dogs

In the first study, four beagle dogs weighing 10.9–12.0 kg were used. All dogs were fasted overnight before dosing a 500-mg tablet. A two-way crossover design with 1-week dosing intervals was used for two selected formulations containing HPMC of different viscosities (B and C). Drug was orally administered followed by 30 mL of water. Food was returned after the 6-h blood sample was taken. Serial blood samples were collected up to 12 h after single oral dosing.

The second study was carried out to assess the in vivo performance of three tablet formulations with differing concentrations of the same HPMC polymer, Methocel K15M. Twelve beagle dogs were fasted overnight before dosing. A crossover design with 1-week dosing intervals was used for three formulations (B, F, and G). Drug was orally administered followed by 100 mL of water. Food was returned after the 6-h blood sample was taken. Serial blood samples were collected up to 24 h after single oral dosing.

Plasma samples were assayed for valproate by a TDx® fluorescence-polarized immunoassay using an automated TDx® system.

Single-Dose and Multiple-Dose Studies in Humans

A single-dose randomized crossover study was used to evaluate two prototype formulations (A and B) in a group of 15 healthy subjects tested under nonfasting conditions using the marketed enteric tablet as a reference. Each subject received a 500-mg dose as one tablet.

Based on the result of the first single-dose study, Formulation B was further evaluated in a group of 18 subjects in a multiple-dose study. A three-way crossover design was used for comparing the controlled-release tablet formulations tested under nonfasting and fasting conditions with the marketed enteric tablet, Deapkote®, under fasting condition. Each subject received a 1000-mg dose as two controlled release tablets taken once daily, and a 500-mg dose as one reference tablet taken twice daily for up to 6 days.

The above studies in humans were designed and carried out by the Department of Drug Metabolism of Abbott Laboratories. Blood samples were collected over 72 h after each drug administration. Plasma samples were assayed for valproate content using a sensitive and specific gas chromatographic procedure.

Data Analysis

Pharmacokinetic parameters were estimated by noncompartmental techniques. Peak drug concentration (C_{max}) and the time to peak drug concentration (t_{max}) were obtained directly from the data without interpolation. The area under the plasma concentration–time curve (AUC_{0–t}) was calculated by the trapezoidal rule. Terminal phase rate constant was obtained by log-linear regression of the terminal phase data for estimation of AUC_{0–∞}. AUC_{0–t} is the sum of AUC_{0–t} and AUC_{t–∞} with the latter computed as the last nonzero concentration divided by the terminal rate constant. The two one-sided hypotheses at significance level (α) of 0.05 were tested for bioavailability by constructing the 90% confidence intervals from natural logarithms of AUC of the test formulations relative to the reference.

RESULTS AND DISCUSSION

Formulation Design

Feasibility of controlled oral delivery of a compound is often dictated by its physicochemical, biopharmaceutic and therapeutic properties, as well as physiological constraints. Based on the physicochemical and biopharmaceutic properties of divalproex sodium, controlled release of...
divalproex sodium may be potentially achieved using any of the three types of well-defined controlled-release systems, i.e., matrix, membrane controlled, and osmotic systems. As part of the initial feasibility assessment, pharmacokinetic simulation indicated that an extended in vivo absorption of at least 20 h was necessary to achieve once-daily dosing, suggesting that absorption of a significant portion of the total dose in large intestine is required. In the present study, matrix systems are preferred due to their effectiveness and robustness as well as the high dose required for divalproex sodium. In addition, hydrophilic matrices were used to control the drug release rate not only because of its use of conventional production methods and lower cost, but also because of the increased chance for complete absorption in the lower portion of the gastrointestinal (GI) tract where limited fluid is available for dissolution.

**In Vitro Drug Release**

*In vitro* drug release from matrices containing different hydrophilic polymers at various concentrations are shown in Figure 2. Variability in release data was found to be very low (RSD < 3%). Faster release was observed from the matrix containing sodium alginate compared with those containing HPMC at the same polymer level. Use of higher concentration or higher viscosity grade of HPMC resulted in slower release. Similar results were obtained in further studies using different concentrations of HPMC as shown in Figure 3. However, it was noted that drug release from formulations containing high viscosity grade HPMC was incomplete at 24 h. In addition, the differentiation of release rates among formulations containing different HPMC concentrations was relatively weak with the *in vitro* test.

The mechanism of drug release from the hydrophilic matrix system involves polymer swelling/erosion and Fickian diffusion of the drug. Release kinetics of hydrophilic systems have been studied extensively, and is dependent upon the solubility, dose, and diffusivity of the drug as well as different characteristics of the rate controlling polymers.8–12 Because divalproex sodium is soluble at neutral pH, drug diffusion through the swollen polymer layer dictates the release kinetics from matrices containing Methocel K15M that has low polymer disentanglement concentration13 (Figure 3). As a result, drug release from Tablets B, F, and G can be described by square-root-of-time relationship (Table 2).

![Figure 2. In vitro release of valproate from controlled-release hydrophilic matrices (B–E, H) tested in the initial formulation study (USP II, 100 rpm, 1-h 0.1 N HCl followed by pH 6.8 phosphate buffer).](image)

![Figure 3. In vitro release of valproate from controlled-release hydrophilic matrices (A–B, F–G) used in in vivo studies (USP II, 100 rpm, pH 6.8 phosphate buffer).](image)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polymer Concentration (%)</th>
<th>Release Rate (% h⁻⁰·⁵)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>30</td>
<td>16.67</td>
<td>0.9961</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
<td>19.18</td>
<td>0.9962</td>
</tr>
<tr>
<td>G</td>
<td>40</td>
<td>14.99</td>
<td>0.9913</td>
</tr>
</tbody>
</table>
**In Vivo Absorption in Dog Model**

Figure 4 shows the results of a preliminary single dose study in dogs of two formulations (B and C) with differing *in vitro* release rates. Plasma concentration profile of an intravenous solution of divalproex sodium from a separate study is also shown as a reference. The estimates of the absolute bioavailability of Formulations B and C, were 56.9 (±13.4)% and 71.1 (±10.8)% respectively. Both formulations exhibited rapid initial absorption followed by extended absorption with overall higher rate of absorption for Formulation C ($C_{\text{max}} = 46.5 \pm 10.4 \, \text{mg/ml}$) when compared with Formulation B ($C_{\text{max}} = 26.2 \pm 6.3 \, \text{mg/ml}$). Although an immediate release formulation was not included in the study, rapid and complete absorption of valproate from such dosage form has been reported by Bialer et al. with an average $t_{\text{max}}$ of 30 min and a dose-corrected $C_{\text{max}}$ of 70.5 mg/mL.14,15 The study also demonstrated the same oral absorption profiles of sustained release dosage form of valproate with and without food in dogs.15 Thus, this study was carried out only in fasting dogs. Preliminary study in a dog model can be useful in designing and evaluating controlled-release dosage form. However, there are known differences between human and dog in motility, bacterial metabolism and GI transit time.16 As a result, decreases in the extent of absorption from sustained-release dosage forms of certain compounds, including valproic acid, have been observed due to shorter intestine residence time or different metabolism.16 Because divalproex sodium is well absorbed from the GI tract, reduced bioavailability of two formulations in this study can be primarily attributed to short residence time in dog GI tract. The same formulations are expected to show higher extent of absorption in humans. In situations like this, evaluation of control of the rate of absorption by dosage forms is more important when dog is used as a model.

Based on the first study, three matrix formulations with faster, medium, and slow release (F, B, and G) were tested in dogs to evaluate the relationship between *in vitro* release and *in vivo* absorption. Figure 5 shows the drug plasma concentration–time profiles following single oral administration of each formulation in dogs. To test for dissolution-controlled absorption and directly evaluate extended *in vivo* absorption,7 plasma concentration data following i.v. administration (from a separate study) were fitted to polyexponentials (RSTRIP, Micromath18, Salt Lake City) and used as unit impulse response $C_d(t)$. Drug plasma data from tablet formulations $C(t)$ were fitted to a smoothing cubic spline function and then deconvoluted with $C_d(t)$ using PCDCON (W.R. Gillespie) to estimate the apparent *in vivo* drug absorption profiles from the tablet formulations (Figure 6).7 Extended *in vivo* absorption of approximately 10–15 h was obtained with three formulations having varying *in vitro* release rates. The same rank order was observed between *in vitro* release rate and *in vivo* rate of absorption, i.e., $F > B > G$. However, the differences in the rate of absorption among three formulations...
appeared to be greater than those in in vitro release rate (Figure 3), suggesting that the in vitro test may not be as discriminating.

**In Vivo Absorption in Humans**

Plasma valproate levels after the 500-mg single doses of Formulations A and B are shown in Figure 7. The tablets were dosed in nonfasting subjects for increased gastric retention. The main caution with such design was that variability in \( t_{\text{max}} \) of the reference enteric tablet is too high to be of practical value. Nevertheless, in this first study in human subjects, prolonged drug absorption was achieved with both formulations. The differences in the curves reflect the different release rates of the two formulations. Average peak concentration (\( C_{\text{max}} = 29.6 \pm 6.7 \) \( \mu \text{g/mL} \)) of Formulation B was significantly lower than that of Formulation A (\( C_{\text{max}} = 40.9 \pm 5.2 \) \( \mu \text{g/mL} \)) and the time to \( C_{\text{max}} \) was significant longer with Formulation B (13.2 versus 7.2 h). Average \( C_{\text{max}} \) of enteric reference tablet was 47.4 (±7.5) \( \mu \text{g/mL} \). Mean AUC values of Formulations A, B, and the reference were 1005 (±261.3), 967 (±371.3), and 1041 (±335.2) \( \mu \text{g h/mL} \), respectively, despite incomplete release in vitro and in vivo absorption in the dog model. Based on the results of this study, Formulation B was chosen for further evaluation of its performance and impact of food intake in a multiple dose study.

Figure 8 shows the steady-state plasma valproate concentration–time profiles following multiple doses of once-daily Formulation B under fasting and nonfasting conditions, and a twice-daily reference enteric tablet under fasting condition. There was no evidence of dose dumping or loss of rate control with Formulation B. Plasma levels were well controlled, and remained nearly constant within therapeutic windows (50–100 \( \mu \text{g/mL} \)) during the 24-h dosing interval, which approximates the response from a constant input. Both peak concentration and peak time of Formulation B was statistically significantly different from those of the reference under fasting conditions (\( C_{\text{max}} = 80.5 \pm 18.6 \) versus 99.4 ± 15.7 \( \mu \text{g/mL} \), \( t_{\text{max}} = 13.6 \pm 6.3 \) versus 3.6 ± 0.9 h). Average AUC
values of Formulation B under fasting and nonfasting conditions were 1592 (±402) and 1709 (±276) μg h/mL, respectively, compared to 1789 (±332) μg h/mL with the reference. The 90% confidence intervals based on the natural logarithm of AUC_{0–24} for Formulation B were 81.7–97.1% and 89.0–105.8% in the absence and presence of food, respectively. These results indicate that Formulation B is bioequivalent to the reference product without significant impact from food intake.

In Vitro/In Vivo Relationship

Exploring a relationship between the in vivo absorption and in vitro drug release from a controlled-release dosage form is an important part of the dosage form development process.\textsuperscript{17} To assess this type of relationship, the percent drug release in vitro was compared with the percent absorption in dogs at a given time point for three tablet formulations (B, F, and G) designed to provide faster, medium, and slow drug release. Good linear relationships were observed for all three formulations (Figure 9 and Table 3). Nearly identical 1:1 correlation was obtained with Formulation B and G. However, it was noted that the correlation for faster-releasing Formulation F deviated significantly from the relationship obtained with the slower-releasing formulations. A slope of 0.70 with an intercept of 20.5 also suggested a possible nonlinear relationship with higher in vivo absorption rate than in vitro release rate during early phase of absorption.

In evaluating the relationship between in vitro and in vivo data from human studies, in vivo apparent absorption profiles from the single dose study were calculated for Formulation A and B using the Wagner-Nelson method. It was found that, unlike in vitro release, in vivo absorption followed a near-zero-order kinetics for Formulation B. The absorption profiles versus percent release in vitro at the same time point are shown in Figure 10 and Table 3. Slopes greater than 1 suggest more rapid in vivo absorption compared with in vitro release, which is consistent with the discrepancy in rate and extent found in the dog model. Negative intercept values may be attributed to lag time in drug absorption due to dosing under nonfasting condition. Although apparent linear relationships were obtained with both formulations, the relationships are clearly formulation dependent and exhibited a certain degree of curvature that is likely a direct result of the difference in release kinetics between in vitro and in vivo. Therefore, in vitro release test conditions will need to be altered to develop a single in vitro—in vivo correlation (IVIVC) applicable to formulations with different release rates.

In summary, a one-daily controlled-release tablet dosage form was successfully designed with desired in vivo performance. However, further investigations are required to obtain a validated in vitro—in vivo correlation for this dosage form.

Table 3. Results of Linear Regression between Percent Release In Vitro Versus Percent Absorption In Vivo for Matrices Containing Different Levels of HPMC

<table>
<thead>
<tr>
<th>Subject</th>
<th>Condition</th>
<th>Tablet</th>
<th>Methocel Concentration</th>
<th>Slope</th>
<th>Intercept</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>Fasting</td>
<td>B</td>
<td>30% K15M</td>
<td>1.02</td>
<td>−1.99</td>
<td>0.9747</td>
</tr>
<tr>
<td>Dog</td>
<td>Fasting</td>
<td>F</td>
<td>20% K15M</td>
<td>0.70</td>
<td>20.52</td>
<td>0.9417</td>
</tr>
<tr>
<td>Dog</td>
<td>Fasting</td>
<td>G</td>
<td>40% K15M</td>
<td>0.95</td>
<td>−0.36</td>
<td>0.9926</td>
</tr>
<tr>
<td>Human</td>
<td>Nonfasting</td>
<td>B</td>
<td>30% K15M</td>
<td>1.73</td>
<td>−20.28</td>
<td>0.9811</td>
</tr>
<tr>
<td>Human</td>
<td>Nonfasting</td>
<td>A</td>
<td>18% K100LV + 8% K15M</td>
<td>2.67</td>
<td>−45.98</td>
<td>0.9518</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

Technical assistance from Lisa Ruiz is appreciated. The authors also would like to thank Drs. Alex Chun and Emil Samara for supplying data from human studies, and Dr. W. Gillespie for providing the PCDCON program.

REFERENCES
