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EFFECT OF VIGABATRIN AND GABAPENTIN ON PHENYTOIN PHARMACOKINETICS IN THE DOG

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This study was aimed at investigating whether or not the kinetics of intravenously administered phenytoin (PT) was altered by oral administration of vigabatrin (VGB) or gabapentin (GBP).

A daily dose of PT (12 mg kg^{-1} i.v.) was given to a group of five beagle dogs for a period of 1 week. On day eight, plasma samples were serially collected over 24 h, after administration of the PT dose. PT administration was continued, along with supplementary oral VGB (60 mg kg^{-1}) for another week and then plasma samples were collected for analysis of PT levels. The same protocol was followed for the PT (12 mg kg^{-1}, i.v.)–GBP (300 mg caps., p.o.) study on a separate group ($n = 5$) of dogs.

Orally administered GBP did not significantly alter the pharmacokinetic parameters of parenteral PT. However VGB markedly changed the drug's kinetics, as evidenced by a 31% ($P = 0.015$) reduction in total body clearance (CL) and an increase of over 45% in half-life ($t_{1/2}$), ($P = 0.013$) and area under the plasma PT concentration–time curve (AUC), ($P = 0.044$).

GBP does not appear to have any pharmacokinetic interaction with PT, while coadministration of VGB and PT results in a marked reduction in systemic clearance of the latter in the dog.

KEY WORDS: phenytoin, vigabatrin, gabapentin, pharmacokinetic interaction, dogs.

INTRODUCTION

Phenytoin (PT) is considered a first line therapy for the treatment of partial seizures with or without generalization. Vigabatrin (VGB) and gabapentin (GBP) are relatively new adjunct antiepileptic drugs (AEDs), which can be coadministered to patients with refractory epilepsy receiving PT medication.

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that acts on the postsynaptic membranes of the central nervous system (CNS) to open chloride channels, resulting in membrane hyperpolarization. It is believed to play a major role in the brain's ability to control seizure activity. Following release into the synapse, GABA is degraded by the enzyme GABA-transaminase (GABA-T) [1].

Vigabatrin (VGB), [(±)-4-amino-5-hexenoic acid; γ-vinyl-GABA] is a structural analogue of GABA, Fig. 1. It exists as a racemic mixture of the S(+) and R(−) enantiomers with only the former as the pharmacologically active component [2]. VGB irreversibly inhibits GABA-T to increase brain levels of GABA [3]. Clinically, VGB is effectively used as an adjunctive anticonvulsant for the treatment of multidrug-refractory complex partial seizures in adults. It has also been effective in the management of resistant partial seizures and infantile spasms in both children and adolescents [3].

Following oral administration, VGB is rapidly absorbed with peak concentration being reached within the first 2 h. Its absorption does not appear to be altered by food [4]. Since VGB is negligibly bound to plasma proteins and is hydrophilic in character, it is widely distributed in the body with an apparent volume of distribution of 0.8 l kg^{-1} [4]. About 70% is eliminated from the body, mainly as the unchanged drug, through renal excretion. It is assumed that hepatic and extrahepatic degradation account for the remaining 30% of an administered dose [4, 5].

Gabapentin (GBP), [1-(aminomethyl) cyclohexaneacetic acid], Fig. 1, is also a GABA analogue but with much improved penetration of the blood–brain barrier. It

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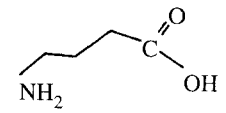
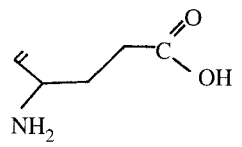
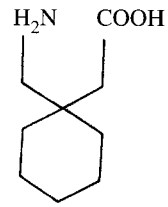


γ—aminobutyric acid (GABA



Vigabatrin (γ—vinyl—GAB/



Gabapentin

Fig. 1. Chemical structures of GABA, vigabatrin and gabapentin.

is currently indicated as adjunctive therapy for the treatment of partial seizures with or without generalization in adult patients [6]. The precise mechanism of the central action of GBP remains unclear, but it is speculated that it increases GABA synthesis [7]. GBP is well absorbed orally, with peak serum levels occurring within 2 h. It circulates mostly unbound in the plasma and is excreted unchanged in the urine without appreciable metabolism in the body. Its absolute bioavailability is approximately 60% and this is not altered by food [4].

Previous studies on the effect of VGB on PT have reported that VGB decreased PT levels by about 20% [8, 9]. However, the authors were unable to elucidate the mechanism for such a decrease. Other studies have shown that VGB has no significant effect on plasma PT concentrations [10, 11]. With respect to GBP, the literature contains conflicting reports. Tyndel [12] indicated that administration of GBP to patients maintained on PT therapy resulted in increased PT levels, leading to toxicity. However, other investigators reported that the addition of GBP to PT monotherapy did not significantly affect PT levels [13, 14].

The aim of this study was designed to reassess the interaction potential of VGB and GBP with PT in dogs.

MATERIALS AND METHODS

Materials

Authentic PT powder was purchased from Fluka (Switzerland) while phenytoin sodium (250 mg per 5 ml ampoule, Abbott Labs, USA), the commercial formulation, was locally purchased from a drug store. VGB (Sabril®, 500 mg tablets, Hoechst-Merrell Dow, France) and GBP (Neurontin®, 300 mg capsules, Parke Davis, USA) were locally purchased from a drug store. Internal standard, 9-hydroxymethyl-10-carbamyl acridan was kindly supplied by Novartis (Basle, Switzerland). Methanol and acetonitrile were of HPLC grade. All other reagents and solvents used were of analytical grade.

Animals

The two studies involving PT with VGB or GBP were performed on two different occasions. A group of five male beagle dogs (USA) weighing between 10 and 14 kg were used in each study. These animals were bred in our Experimental Animal Care Centre (College of Pharmacy, King Saud University, Riyadh, Saudi Arabia).

(i) Phase I:

The dogs received PT (12 mg kg^{-1}, i.v.) daily for a period of 7 days. At the end of this period, the animals were placed in an upright position in a restrainer cage. The leg was shaved and a cannula (18-gauge) placed in the femoral vein. The PT dose was slowly administered intravenously to the dogs. Blood samples (*c.* 1.0 ml) were collected via the cannula just before and 0.25, 0.5, 1.0, 1.5, 2, 4, 6, 9, 12 and 24 h after drug administration. After each sample withdrawal, the cannula was flushed with an equal volume of heparinized saline. The blood samples were then immediately centrifuged at 1000 ***g*** for 10 min and the plasma samples were aspirated and kept frozen at −20 °C pending analysis.

(ii) Phase II:

This phase of the study started immediately following collection of the last sample of phase I. The animals of phase I started receiving VGB (60 mg kg^{-1}, p.o.) or GBP (300 mg caps., p.o.) 1 h before PT (12 mg kg^{-1}, i.v.) administration, for a period of 1 week. At the end of this period, the blood samples were obtained using exactly the same procedure as in phase I.

Drug analysis

Plasma PT samples were measured by the previously reported method of high performance liquid chromatography [15]. This method was able to detect PT in plasma sample concentrations as low as 0.2 $\mu g\ ml^{-1}$. The inter-assay coefficients of variation (%CV) ranged between 1.2 and 4.8%.

Briefly, the sample procedure involved the following. To 100 μl of plasma sample, 20 μl of internal standard (9-hydroxymethyl-10-carbamyl acridan; 50 $\mu g\ ml^{-1}$) was added and then extracted with 100 μl of diethyl ether. The mixture was vortex-mixed for 30 s, shaken in a rotary mixer for 5 min and centrifuged at 1000 *g* for 10 min. The organic layer was separated and evaporated to dryness. The residue was redissolved with 100 μl of mobile phase and subsequently 20 μl of this sample was injected onto the chromatograph.

Pharmacokinetic and statistical analysis

Phenytoin pharmacokinetic parameters were determined by non-compartmental methods [16]. The first-order elimination rate constant (K_{el}) was determined from the slope of the best log-linear fit of the terminal phase by least-squares linear regression analysis. Elimination half-life ($T_{1/2}$) was calculated as $0.693/k_{el}$. Area under the plasma concentration–time curve (AUC) and area under the first moment of the plasma concentration–time curve (AUMC) were calculated by the linear trapezoidal rule with extrapolation to time infinity. Mean residence time (MRT) was calculated from the equation, $MRT = AUMC_{0-\infty}/AUC_{0-\infty}$. Total body clearance (CL) was calculated as $CL = Dose/AUC_{0-\infty}$ and consequently volume of distribution (V_d) was calculated as $V_d = CL/K_{el}$. The pharmacokinetic parameters are presented as mean ± SD. Treatment effects were evaluated by paired *t*-tests with $P \leq 0.05$ as the level of significance (STAT 100, version 1.24, 1995, Biosofot, Cambridge, UK).

RESULTS

The influence of concomitant administration of VGB on PT disposition is demonstrated in Fig. 2 and Table I, while that of GBP is depicted in Fig. 3 and Table II. Although the pharmacokinetic parameters presented in Tables I and II (with regard to PT alone) are from two different groups of animals, these showed highly consistent results, suggesting the reliability of the data and the methods used to obtain them.

As illustrated in Fig. 2, VGB had a marked influence on the plasma concentration–time profile of intravenously administered PT. At almost all sampling times, plasma PT levels were considerably higher in the presence of VGB as compared to baseline values. Computed mean pharmacokinetic parameters (Table I) showed that VGB caused a decrease of 31% ($P = 0.015$) in PT total body clearance (CL) with a corresponding increase of 49% ($P = 0.044$) in total area under the plasma PT concentration–time curve (AUC). Furthermore, the terminal half-life ($t_{1/2}$) and mean residence time (MRT) were prolonged by 45% ($P = 0.013$) and 53% ($P = 0.016$), respectively. The volume of distribution (V_d) of PT remained unaltered ($P = 0.859$) during treatment with VGB.

Table I
Mean (± SD) pharmacokinetic parameters of PT (12 mg kg^{-1}, i.v.) in dogs before and after VGB (60 mg kg^{-1}, p.o.) administration ($n = 5$)

Parameter	*Before*	*After*	*P-value*
$T_{1/2}$ (h)	1.89 ± 0.11	2.74 ± 0.36	0.0126[a]
$AUC_{0-\infty}$ (mg h l^{-1})	29.57 ± 2.54	44.14 ± 12.02	0.0440[a]
MRT (h)	2.60 ± 0.12	3.97 ± 0.69	0.0162[a]
V_d(l kg^{-1})	1.14 ± 0.15	1.16 ± 0.22	0.8587
CL (l h^{-1} kg^{-1})	0.42 ± 0.03	0.29 ± 0.07	0.0152[a]

[a] Significantly different from baseline (before), using a paired *t*-test ($P \leq 0.05$).

Table II
Mean (± SD) pharmacokinetic parameters of PT (12 mg kg^{-1}, i.v.) in dogs before and after GBP (300 mg, p.o.) administration ($n = 5$)

Parameter	*Before*	*After*	*P-value*
$T_{1/2}$ (h)	1.66 ± 0.35	1.94 ± 0.45	0.3894
$AUC_{0-\infty}$ (mg h l^{-1})	28.28 ± 10.73	33.22 ± 10.20	0.3863
MRT (h)	2.31 ± 0.46	2.77 ± 0.73	0.3508
V_d(l kg^{-1})	1.07 ± 0.22	1.06 ± 0.20	0.9402
CL (l h^{-1} kg^{-1})	0.47 ± 0.15	0.41 ± 0.17	0.5606

[a] Significantly different from baseline (before), using paired *t*-test ($P \leq 0.05$).

On the other hand, treatment with GBP failed to show any statistically significant alterations in all the PT pharmacokinetic parameters (Table II).

DISCUSSION

The data shown in Table II do not support a recent report of Tyndel [12] suggesting a significant interaction between GBP and PT. They confirm, rather, the earlier findings of Anhut *et al.* [13] and Crawford *et al.* [14] who reported a lack of interaction between GBP and PT.

PT is extensively metabolized in the liver to inactive compounds, which are excreted in the urine, with less than 5% of the dose excreted unchanged in the urine. The main metabolic pathway is catalysed by the isoenzymes of cytochrome P-450; CYP2C9 and, to a minor extent, CYP2C19, to form *p*-hydroxyphenytoin (*p*-HPPH), which is then extensively conjugated with glucuronic acid and excreted in the urine [17]. Therefore, known inhibitors of these isoenzymes will decrease the metabolism of PT and consequently result in increased plasma PT levels [18]. Unlike PT, GBP is neither metabolized by liver microsomal oxidase enzymes nor bound to plasma proteins.

With the concurrent administration of VGB and PT, the latter's pharmacokinetic behaviour was significantly

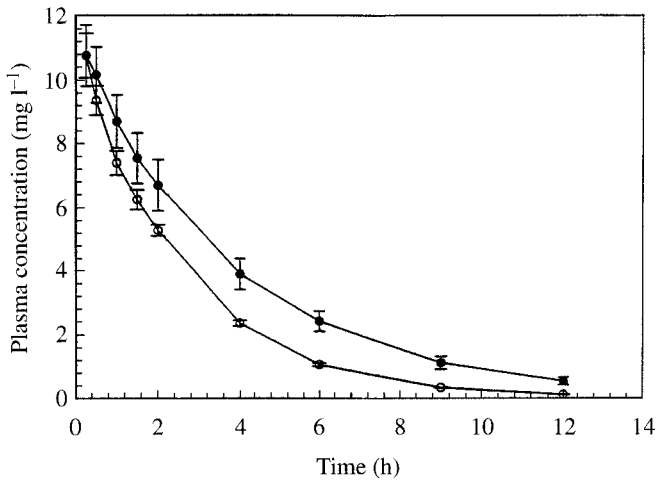


Fig. 2. Mean (±SEM) plasma concentration–time profile of phenytoin (PT), (12 mg kg^{-1}, i.v.) administered alone (○) or after administration with vigabatrin (VGB) (●) (60 mg kg^{-1}, p.o.) in dogs ($n = 5$).

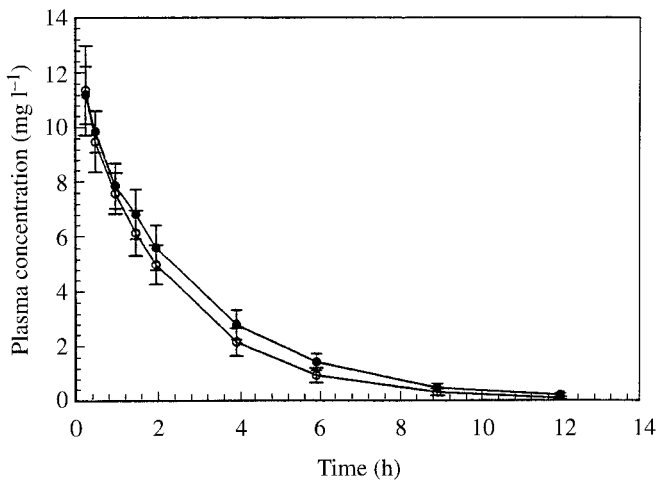


Fig. 3. Mean (±SEM) plasma concentration–time profile of phenytoin (PT), (12 mg kg^{-1}, i.v.) administered alone (○) or after administration with gabapentin (GBP) (●) (300 mg, p.o.) in dogs ($n = 5$).

altered. As shown in Table I, the mean PT total body clearance (CL), elimination half-life ($T_{1/2}$), MRT and the total area under the plasma PT concentration–time curve (AUC) were all influenced by VGB. The only parameter that remained largely unaltered was the apparent volume of distribution (V_d). The most probable mechanism for such a pharmacokinetic interaction is a metabolic one, at the level of microsomal enzymes. This is very likely since the total body clearance of PT, most of which is accounted for by *p*-HPPH, was reduced by 31% in the presence of VGB. By contrast, PT clearance only accounts for less than 5% of the total body clearance.

Moreover, PT is a highly plasma-protein bound AED (>90%). Displacement of PT from its plasma-protein binding sites will result in an increase in the drug's apparent volume of distribution accompanied by a decrease in the total plasma concentration [18]. The displacement of PT from plasma-protein binding by VGB is unlikely because VGB itself is poorly bound to plasma proteins and the value for the mean apparent volume of distribution during coadministration is unaltered (Table I).

Our findings do not concur with the reports of Rimmer and Richens [8]. These investigators reported seeing unaltered plasma PT levels compared to baseline values until the fourth week of concurrent VGB therapy, when a decrease of 23% occurred in plasma PT levels of epileptic patients. This is further confirmed by the apparent lack of change in the value of the mean plasma PT levels, suggesting poor compliance to be the possible explanation for the previous findings [19]. Limitations of the Rimmer and Richens' [8] findings were that the plasma PT samples were analysed using a non-specific method (fluorescence polarization immunoassay; FPIA). Compared to the present HPLC method, FPIA is less specific and prone to interference with the drug's metabolites [20]. Also, the investigators did not provide any pharmacokinetic data for PT either before or after coadministration of VGB.

The few studies to date on the fate of VGB enantiomers have reported that VGB is weakly metabolized with 70% eliminated unchanged in the urine. Urinary recovery of the S(+) enantiomer, the active isomer, is only 49% compared to the inactive one, R(−), where recovery is 65% [4].

Although VGB is weakly metabolized, as reported by some investigators [5] without determining the exact percentages of enantiomers metabolized, it has been noted that VGB maximum plasma concentrations are lowered in patients taking enzyme-inducing drugs, which suggests some involvement of hepatic metabolism of VGB [5].

The induced microsomal system is more susceptible to inhibitory influences by coadministered agents [21]. In this regard, it has been shown that ranitidine inhibits hepatic cytochrome P-450 enzymes of many drugs, although it is a less potent inhibitor than cimetidine. Hoensch *et al.* [21] suggested that ranitidine may be as potent as cimetidine in individuals whose hepatic cytochrome P-450 has been induced and therefore isoenzymes of P-450 present in the induced state may be more subject to inhibition by ranitidine than in the non-induced state. This hypothesis may explain why the dogs in this study, which had hepatic induction caused by PT, were more susceptible to hepatic inhibition from VGB leading to a reduction in total body clearance and an increase in the elimination half-life. Moreover, the probable mechanism for the effect of VGB on PT pharmacokinetics in this species appears to be inhibition of the cytochrome P-450 isoenzymes (CYP2C9 and CYP2C19) leading to a reduction in PT metabolism. Further studies employing the pharmacologically active enantiomer, S(+), of VGB may clarify the exact nature and degree of the proposed pharmacokinetic interaction.

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