Chapter

Active Pharmacovigilance in Epileptic Patients: A Deep Insight into Phenytoin Behaviour

Marta Vázquez, Pietro Fagiolino, Cecilia Maldonado, Natalia Guevara, Manuel Ibarra, Isabel Rega, Adriana Gómez, Antonella Carozzi and Carlos Azambuja

Abstract

Despite the introduction of new and more expensive anticonvulsant drugs, phenytoin (PHT) is still a first-line medication for common types of epilepsy such as tonic-clonic and complex partial seizures but not for absence seizures. PHT shows a nonlinear kinetics and a narrow therapeutic range, thus a fine balance must be found between efficacy and toxic effects. Since the free (unbound) drug is responsible for producing the pharmacological effect, the concentration in a novel biological fluid more closely related to arterial free plasma drug concentration—saliva—is used in this study as part of the monitoring strategy. Therefore, in order to optimize therapy in epileptic patients under PHT treatment, plasma and saliva concentrations of PHT were measured, and adverse drug reactions were registered during a 2-year follow-up. CYP2C9, CYP2C19, and epoxide hydrolase polymorphisms (enzymes involved in PHT metabolism) were also analyzed using, in this way, pharmacogenetics for drug safety. The two PHT brands commercially available in our country and used in this study demonstrated similar pattern of efficacy and safety.

Keywords: phenytoin, pharmacovigilance, therapeutic drug monitoring, pharmacokinetics, pharmacogenetics

1. Introduction

Phenytoin (PHT) is approved to be used for almost any type of epilepsy such as generalized tonic-clonic and complex partial (psychomotor and temporal lobe) seizures and for preventing and treating seizures occurring during or following neurosurgery except for absence seizures [1]. Regarding its mechanism of action, PHT exerts a stabilizing effect on excitable membranes of several cells, including neurons and cardiac myocytes. It inhibits voltage-dependent sodium channels, reducing sodium flow during action potential [2]. Taking into account, PHT has a narrow therapeutic range; therapies with this drug are monitored by plasma quantification in the routine practice [3]. However, when using plasma drug concentrations in therapeutic drug monitoring (TDM), free plus protein bound drug is measured. As it is known, plasma or serum concentration does not usually

represent the concentration at its receptor site. Only free drug can reach the biophase (action site) and interact with a receptor to produce the effect (therapeutic or toxic). Total drug concentrations depend on protein binding, and PHT is highly bound to albumin. But, free drug monitoring is rather expensive for routine practice. Our research group has been working for several years using saliva as biological fluid [4–6]. Saliva is not only useful for being a simple and noninvasive collection method but also for the information it gives. Studies have demonstrated that salivary concentrations highly correlate with free drug concentration in plasma mainly for drugs, which are lipophilic and nonionized at salivary pH (i.e., phenytoin or carbamazepine), and therefore, saliva concentrations are more reflective of the concentration at the biophase. Saliva is produced in the salivary glands by ultrafiltration of arterial plasma. Arterial drug concentration is higher than the respective venous concentration during drug input either after intravenous or oral administration [7]. So if enterohepatic or blood-gastrointestinal cycling processes are operating, elevated saliva drug concentrations (reflecting higher arterial drug concentrations) during the elimination phase could predict re-entry processes. Using this fluid, our research group has studied enterohepatic recirculation of paracetamol [8] and blood gastrointestinal cycling of methadone [9].

It is important to note that for the treatment of epilepsy, the effective and safe plasma concentrations referenced in the literature are between 10 and 20 mg/L, and salivary concentrations are between 1 and 2 mg/L. The narrow population therapeutic range, the clinical consequences of presenting subtherapeutic or toxic concentrations, and the difficulty of determining the pharmacokinetic parameters for each patient predispose the clinician to follow the therapy with this anticonvulsant agent through a frequent determination of plasma and/or salivary concentrations in each patient. Therefore, the dose range compatible with a therapeutic serum or a saliva concentration is narrow within subjects, and TDM is of particular value in dosage tailoring [3, 10].

PHT is a weak acid, which usually administered as the sodium salt. Its solubility in water is scarce even in the intestine as it precipitates at the intestinal pH, a fact that conditions its entry into the body. However, it is well absorbed when administered orally, with a bioavailability close to 90%, which implies that in addition to the great lipophilicity that it presents, the metabolism at the enterocyte level is low. The drug has a volume of distribution of 0.64 L/kg and is approximately 90% bound to plasma proteins [10, 11]. PHT main biotransformation route is para-hydroxylation to form the inactive metabolite 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH). The enzyme involved in this step is CYP2C9 and, to a lesser extent, CYP2C19 [11]. p-HPPH formation occurs via an arene-oxide intermediate, and the accumulation of the latter can be the cause of skin reactions associated with PHT [12, 13]. Further oxidation of p-HPPH leads to catechol formation (3′-4’-diHPPH), by CYP2C9, CYP2C19, and CYP3A4, also undergoing an arene-oxide intermediate. On the other side, the arene oxide can also be converted to transdihydrodiol phenytoin via microsomal epoxide hydrolase (EPXH), which can also lead to catechol formation. The enzymes CYP2C9, CYP2C19, and EPHX are polymorphically expressed [14–16].

There is strong evidence that PHT undergoes an important secretion from blood to the digestive tract after which the drug would re-enter the body from the intestinal lumen. Observations of secondary peaks in plasma concentration profiles after intravenous doses of PHT constitute strong arguments to affirm the important contribution of recirculation between internal medium and gastrointestinal lumen in the pharmacokinetics of this drug [17–19].

PHT was traditionally believed to saturate the hepatic enzymes and thereafter giving rise to a nonlinear concentration-dose relationship, described by

the Michaelis-Menten equation. An alternative hypothesis based on its ability to induce efflux transporters was reported by our group, and this inductive effect was demonstrated in rats to be time-and-concentration-dependent [20–22]. The efflux transporter involved would be MRP2, and by means of this efflux pump, the drug would be secreted, in an important way, towards the digestive tract, thus propitiating the appearance of secondary peaks even after its intravenous administration. The induction of its expression would slow the oral absorption of PHT, a fact that is noticeable when analyzing the plasma profiles of the drug during the interval of administration in multiple doses, which are much less acute than the profiles observed after single doses. On the other hand, the deviation of the drug from the liver to the intestine would avoid the major biotransformation that takes place in the hepatocyte. Given the greater abundance of CYP2C9 and CYP2C19 enzymes present in the liver in relation to the enterocytes, a reduction in clearance in a concentration-dependent manner as verified during chronic treatments could be observed. In other words, the nonlinear kinetics found for this drug would not correspond to an enzymatic saturation but to an induction in the expression of transporters that remove molecules from the sites of high metabolism [20, 22].

The toxic effects of PHT depend on the route of administration, the duration of exposure, and the dose [23]. When administered IV with an excessive speed for status epilepticus, the most important toxic signs are cardiac arrhythmias with or without hypotension and central nervous system depression. These complications are reduced with the slow administration of diluted solutions of the drug. IV administration of PHT should not exceed 50 mg/minute for adults and should be well diluted in physiological solution in order to reduce local venous irritation due to the alkalinity of drug solutions [24–26].

The toxic effects associated with chronic treatment include concentration-related effects, as well as confusion, behavioral disturbances, increased frequency of seizures, gastrointestinal symptoms, hirsutism, gingival hyperplasia, osteomalacia, and megaloblastic anemia. Undesirable effects include vertigo, ataxia, headache, diplopia, and nystagmus but not sedation. Increased incidence of fetal malformations (mainly cleft palate) has been observed in children born from epileptic mothers under PHT treatment. Occasionally, increased hepatic transaminases are also observed [27].

The reported endocrine effects are diverse. Osteomalacia with hypocalcemia and increased alkaline phosphatase has been attributed both to an increased vitamin D metabolism due to the inducing effect of PHT and to inhibition of intestinal calcium absorption. PHT also increases the metabolism of vitamin K and reduces the concentration of vitamin K-dependent proteins that are important for the normal metabolism of calcium in bone [28].

Hypersensitivity reactions can vary from mild rashes in 2–5% of patients to sometimes more severe and life-threatening skin reactions such as Stevens-Johnson syndrome [29].

Active pharmacovigilance, in contrast to passive, seeks to ascertain the number of adverse reactions (lack of efficacy or toxicity) via a continuous process. An example of active surveillance is the follow-up of patients treated with a particular drug. In general, it is more feasible to get more comprehensive data from the particular drug behavior through an active pharmacovigilance system than through a passive reporting system.

Nowadays, two commercial brands of PHT are available in Uruguay for oral administration, both multisource drug products (Antepil® and Comitoína®). Due to the characteristics of PHT previously mentioned, there is a need to evaluate these products in their natural clinical setting because the clinical response is never assessed in bioequivalence studies carried out with healthy volunteers.

2. Objective

The objective of this study was to optimize PHT therapeutics by active pharmacovigilance in epileptic patients under PHT treatment (either Antepil® or Comitoína®) measuring plasma and saliva concentrations, determining CYP2C9, CYP2C19, and epoxide hydrolase (EPHX) polymorphisms, registering adverse drug reactions (ADR) during a 2-year time period, and demonstrating that both PHT brands have similar pattern of efficacy and safety.

3. Patients and methods

3.1 Subjects and design

The inclusion criteria for patients included a diagnosis of epilepsy carried out by the attending neurologist. Some patients were treated only with Antepil® for the study period of 2 years, and some were treated only with Comitoína® during the same period. Patients with hepatic or renal impairment, pregnant and lactating women, and individuals with history of alcohol or drug abuse or addiction or with diminished intellectual or motor abilities were excluded from the study. The study protocol was approved by the Institutional Ethics Review Committee of the Faculty of Chemistry, Universidad de la República, and written consent was obtained from all subjects prior to their participation in the study.

All the information about PHT concentrations in plasma and saliva, PHT dose and dosing interval, comedications, adverse effects (lack of efficacy or toxic effects), and laboratories results (liver and renal function and hemogram tests, albumin in blood) of the patients were collected by the pharmacists of the program using a data sheet elaborated for this purpose every time the patient came for the interview and for blood and saliva analysis (see Section 3.2).

One electroencephalogram in the 2-year period was also obtained.

Patients also had a form in which they wrote down any ADR they experienced, indicating start time and duration. Patients called the neurologists of the pharmacovigilance program when an ADR occurred and the physician determined the action to take and communicated this action to the pharmacists. All the ADRs were notified to the Pharmacovigilance Unit of the Health Ministry completing the Yellow Form. The causality assessment of ADRs was carried out using Naranjo's algorithm as shown in **Table 1**.

3.2 Sampling and chemical analysis

The pharmacovigilance protocol includes predose blood and saliva samples every 3 months and salivary curves when the subject was included in the study and 1 year later. Laboratory tests were performed twice in the 2-year period except for albumin in blood that was carried out in every occasion.

Predose blood samples (5 mL) were taken from the antecubital vein and placed into heparinized tubes, and saliva samples were obtained by stimulation with citric acid and scheduled before dose intake and at 1, 2, 3, 4, 5, 6, 8, and 12 hours after dosing. Blood and saliva samples were centrifuged and stored in freezer (−25°C) until analysis.

Quantification of PHT in saliva was carried out by Chemiluminescent Microparticle Immunoassay (CMIA), using Architect (Abbot™) equipment, according to the instructions given in the package insert. Precision and accuracy were below

Question	Yes	No	Do not know	Score
1. Are there previous conclusive reports on this reaction?	+1	0	0	
2. Did the adverse event appear after the suspected drug was administered?	+2	−1	0	
3. Did the adverse event improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0	
4. Did the adverse event reappear when the drug was readministered?	+2	−1	0	
5. Are there alternative causes that could on their own have caused the reaction?	−1	+2	0	
6. Did the reaction reappear when a placebo was given?	−1	+1	0	
7. Was the drug detected in blood or other fluids in concentrations known to be toxic?	+1	0	0	
8. Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0	
9. Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	
10. Was the adverse event confirmed by any objective evidence?	+1	0	0	
			Total score:	
Total score: >9 = definitive reaction, 5–8 = probable, 1–4 = possible, ≤0 = doubtful.				

Table 1.
Naranjo's casuality algorithm.

15% and between 85 and 115%, respectively, except at the lower limit of quantification (0.3 mg/L), where intra- and inter-day coefficient of variation rose up to 20%.

Plasma PHT and p-HPHH concentrations were determined by a high performance liquid chromatography (HPLC) method based on a procedure previously published by Savio et al. [30]. The method was linear between 0.5286 mg/L (the lower limit of quantification, LLOQ) and 24.39 mg/L for PHT and between 0.0585 mg/L (LLOQ) and 2.701 mg/L for p-HPPH.

3.3 Pharmacokinetic and statistical analysis

The following pharmacokinetic parameters at steady state (ss) were determined from the experimental salivary PHT concentration curves versus time:

- Area under the saliva PHT concentration-time curve from 0 to T hours (AUCss 0–T) calculated by the linear trapezoidal rule with T being the administration interval.
- Experimental maximum and minimum concentration (Cmaxss and Cminss) of the curve.
- Time to obtain maximum concentration (Tmaxss).
- Mean concentration (Cmeanss = AUCss 0–T/T).
- Peak-to-trough fluctuation [PTF = (Cmaxss − Cminss) × 100/Cmeanss].

The statistical processing of the information was carried out using the statistical program Statistical Package for the Social Sciences, version 17 (SPSS), and it was by obtaining mean values, standard deviations, and 95% confidence intervals (95% CI) of the pharmacokinetic parameters.

3.4 Genotyping procedure of EPHX, CYP2C9, and CYP2C19

Genotyping procedure of EPHX, CYP2C9, and CYP2C19 was carried out by Genia (Molecular Genetics Laboratory, Montevideo, Uruguay). EPHX polymorphism was done by real-time polymerase chain reaction (RT-PCR). To determine CYP2C9 and 2C19 genotype, a conventional PCR was performed for each SNP (rs 1799853 and rs 1057910 for CYP2C9; rs 4244285 and rs 4986893 for CYP2C19). The complete technique is specified in the study performed by our group [31].

3.5 *In vitro* dissolution study

Six units of each product were tested in Distek® Dissolution System 2100C equipment. The conditions were as follows: USP 32 Apparatus 2 (paddle); 75 rpm stirring speed; volume 900 mL of water; and temperature 37 ± 0.5°C. Samples were automatically withdrawn by the use of an Agilent 89092EO pump at 10, 15, 20, 30, 40, 60, and 80 minutes. The drug release at different time intervals was measured by UV-visible spectrophotometer (Agilent 8453 and ChemStation® software).

4. Results and discussion

A total of 57 adult Caucasian epileptic subjects were included in the active pharmacovigilance program. Thirty-three individuals were receiving a conventional dose of Antepil® 100 mg (Fármaco Uruguayo Laboratory) and 24 of Comitoína® 100 mg (Roemmers Laboratory). The demographic characteristics of the subjects for Antepil® and Comitoína® are summarized in **Tables 2** and **3,** respectively.

The *in vitro* dissolution assay demonstrated a slow release drug rate for both brands as it can be seen in **Figure 1** but with a faster onset for Antepil®. This is in accordance with the saliva concentration-time profiles of both brands as shown in **Figures 2** and **3**. Although the saliva profiles are similar, a delay at the beginning of the absorption can be seen in Comitoína® profile (**Figure 3**).

Several secondary peaks can be observed after diurnal administration of PHT (**Figures 2** and **3**), and this is evidencing the PHT recirculation processes that have already been studied by our group and other researchers as it was stated in Section 1. PHT could be stored in the digestive system organs to be later excreted to the small intestine lumen, from where it can re-enter into the bloodstream. This phenomenon would be favored by the overexpression of efflux transporters at the bile canaliculus caused by PHT, which would accelerate the escape of PHT molecules from hepatocyte that is the main site of drug metabolism by CYP2C9 and CYP2C19,

	Total	**Male**	**Female**
Subjects	33	15	18
Age (years)	46.6 (18–75)	42.0 (18–73)	50.4 (20–75)
Weight (kg)	75.8 (45–140)	75.9 (49–140)	75.7 (45–120)

Table 2.
Demographic characteristics of the patients under Antepil® treatment expressed as mean (95% CI).

deviating it to the intestinal lumen where metabolizing enzyme expression is poor, an adverse from where it would be available to re-entry into the bloodstream. As it can be observed in both figures, there is no important PTF.

Mean plasma protein binding of PHT was 89% for Antepil® and 90% for Comitoína®, which do not differ from the literature (80–90%). The plasma protein binding (PPB) was obtained as follows:

$$PPB = 100 \times (1 - [So]/[Po]). \quad (1)$$

Being So and Po predose saliva and plasma concentration, respectively.

	Total	Male	Female
Subjects	24	12	12
Age (years)	45.8 (18–76)	47.8 (25–76)	44.0 (18–69)
Weight (kg)	76.5 (48–108)	80.1 (54–100)	66.9 (48–108)

Table 3.
Demographic characteristics of the patients under Comitoína® treatment expressed as mean (95% CI).

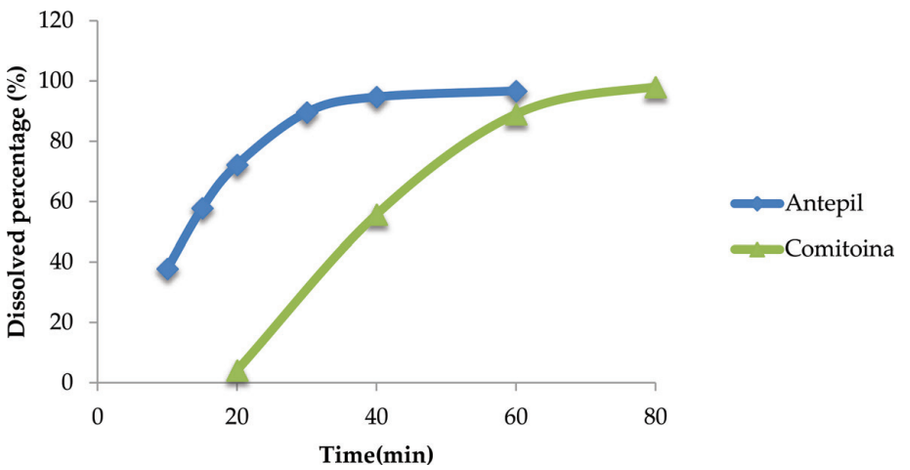

Figure 1.
Dissolution profile of the two brands of phenytoin in water.

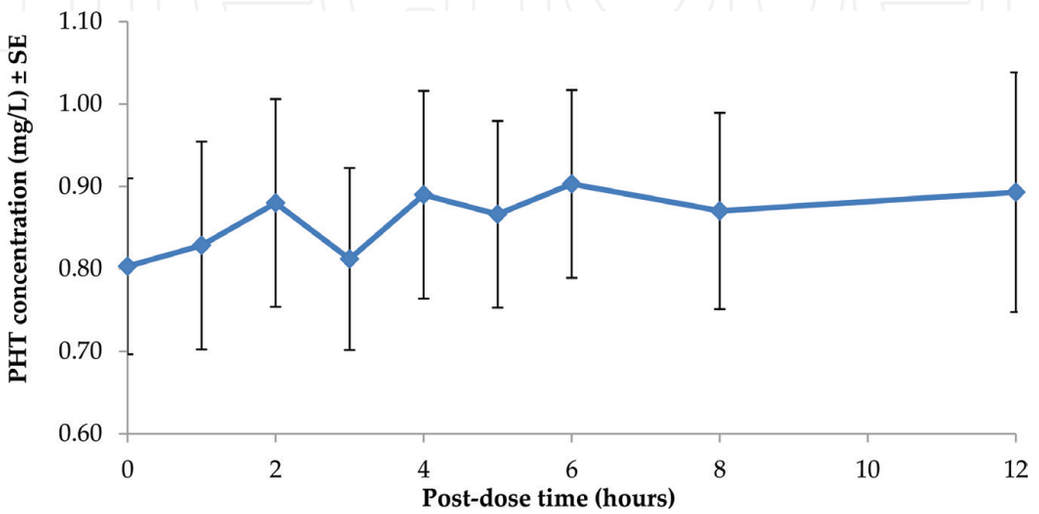

Figure 2.
Mean (±standard error) saliva PHT concentration-time profile after the administration of Antepil®.

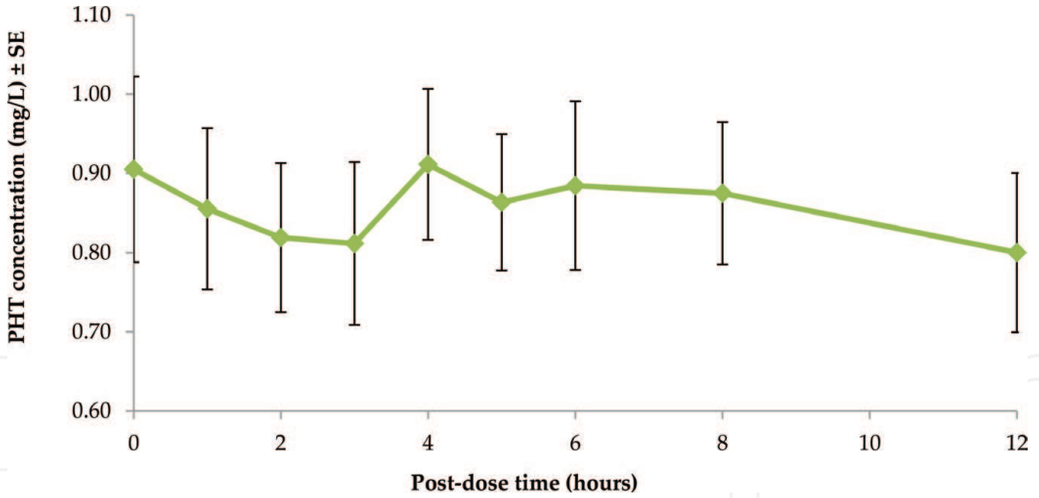

Figure 3.
Mean (±standard error) saliva PHT concentration-time profile after the administration of Comitoína®.

Serum albumin levels were between the reference range (3.30–5.00 g/dL) in all the subjects and in every occasion. No laboratories abnormalities were observed for any subject or brand during the 2-year study, except for ammonia levels determined only in some patients comedicated with valproic acid (VPA).

No significant differences were found in the normalized doses or in the salivary and plasma concentrations between men and women for either brand. However, there is a tendency for women to have lower plasma and saliva PHT concentration for equal normalized doses. Further studies with a greater number of individuals are needed to confirm this tendency. The explanation could be a greater apparent clearance in women due to either a higher systemic clearance of the drug or a lower oral bioavailability. Both could be probably given the greater expression of MRP2 and the higher fraction of cardiac output delivered to the intestine that women present in comparison to men.

Figures 4 and **5** show both plasma and salivary drug concentrations in response to the administered daily doses for Comitoína® and Antepil®, respectively.

Both **Figures 4** and **5** show the typical curvature of Michaelis-Menten kinetics described in the literature for plasma concentrations, considering a limited enzyme capacity. However, as explained in Section 1, an alternative hypothesis could explain the nonlinear kinetics of PHT. The decrease in clearance observed with increasing concentrations would be secondary to the induction of drug secretion from the blood into the intestine, from where it is subsequently reabsorbed. A concentration-local induction dependent on the transport of efflux caused by PHT itself would enhance the processes that prolong its permanence in the splanchnic zone. This would lead to a decrease in the amount of drug metabolized in the liver and a greater percentage of it would enter the process of enterohepatic recirculation, sending the drug to an area of poor metabolism such as the intestine from which it enters the body again. This effect could be responsible for both the low peak-to-trough fluctuation mentioned earlier and the disproportionate increase in plasma concentrations with dose increase.

The most common antiepileptic comedications in the patients included in the study were carbamazepine, lamotrigine, levetiracetam, and valproic acid.

Most adverse reactions with both Antepil® and Comitoína® did not deserve dose reduction according to the neurologist due to the fact that they were related to chronic treatment with PHT. The exception was the appearance of seizures with high PHT concentrations, which was considered as a possible concentration-dependent adverse reaction. PHT concentrations are responsible for the overexpression of

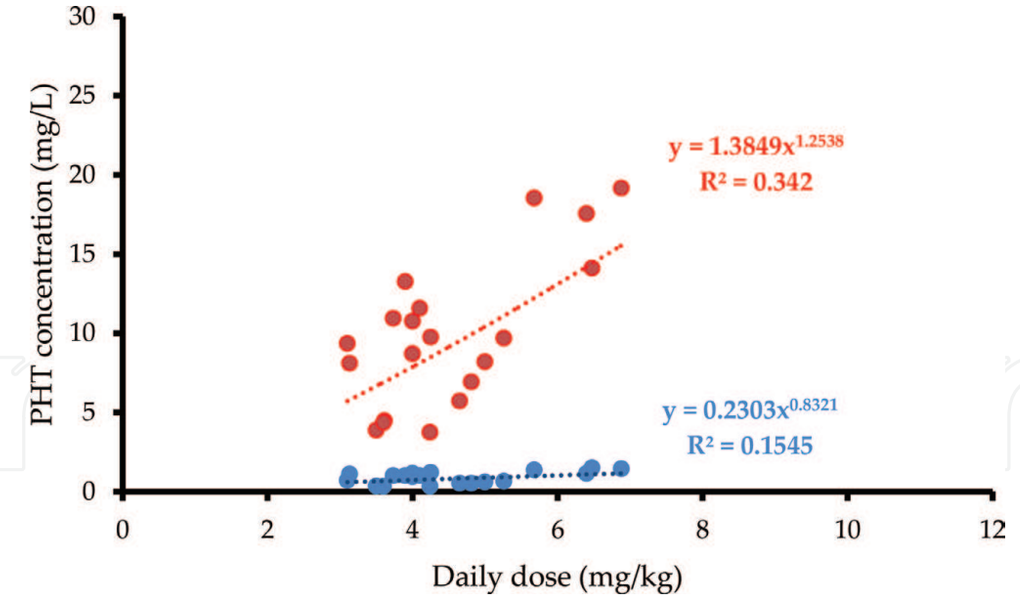

Figure 4.
Relationship between the normalized dose of PHT and plasma concentration (in red) and salivary concentration (in blue) for Comitoína®.

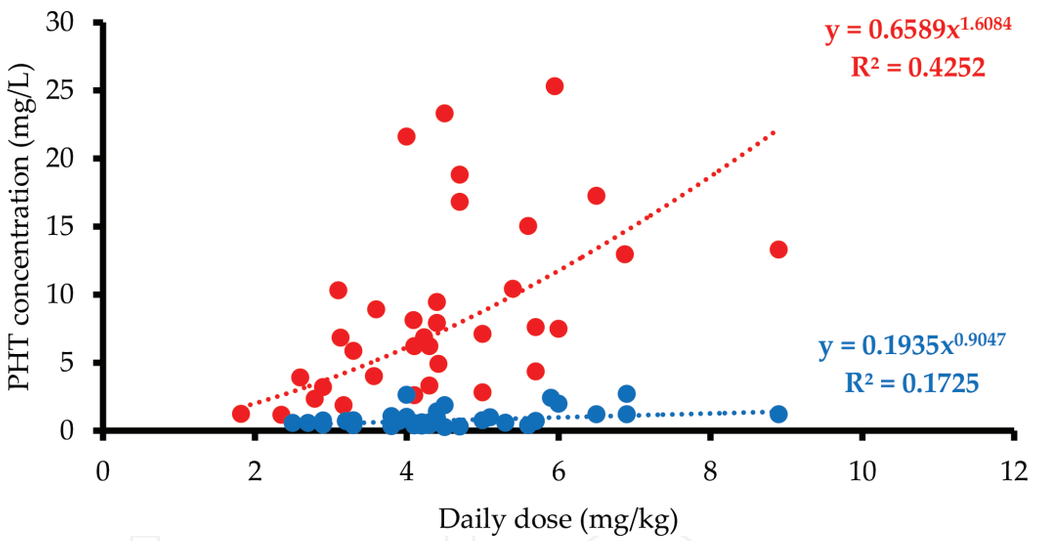

Figure 5.
Relationship between the normalized dose of PHT and plasma concentration (in red) and salivary concentration (in blue) for Antepil®.

efflux transporters, not only in the splanchnic zone but also in the hematoencephalic barrier, which could be the reason for low PHT levels in the brain and the appearance of seizures.

Some patients taking Antepil® were comedicated with VPA. Ammonia levels in some of these patients were higher than the upper limit of the normal range in blood (25–94 μg/dL). Our research group has been studying increased ammonia levels in patients under VPA treatment [32, 33]. High levels of ammonia in the brain can be the cause of seizures as ammonia easily crosses the blood-brain barrier, and in the brain, it can conjugate with α-ketoglutarate to form glutamate. This leads to brain damage and the appearance of seizures given the excitatory activity of glutamate in the synaptic membrane.

Tables 4 and **5** show the detected ADRs in patients under Antepil® or Comitoína® treatment, respectively, and the causality assessment using Naranjo's algorithm.

ADRs	Causality		Total
	Possible	Probable	
Gastrointestinal disorders	1	3	4
Visual problems	0	3	3
Drowsiness	3	0	3
Dizziness	2	1	3
Dysarthria	0	3	3
Bleeding gums/hyperplasia	0	1	1
Memory loss	0	1	1
Tremor in hands	0	1	1
Trouble in sleeping	0	2	2
Lack of strength in lower limbs	2	0	2
Orthostatic hypotension	0	1	1
Walk instability	1	1	2
Seizures*	1	3	4
Total	10	20	30

**Seizures were reported as toxic reactions and not treatment failure when plasma levels of PHT were greater than 20 mg/L and when once the dose was reduced seizures disappear. In one patient, this adverse effect was assessed as possible as the patient was comedicated with VPA (1200 mg) and the ammonia level was 165.8 μg/dL, and as it was explained previously, VPA provoking hyperammonemia could be the cause of the seizures. Predose plasma levels of PHT and VPA were 24.8 and 49.7 mg/L, respectively, in this patient.*

Table 4.
Detected adverse drug reactions (ADRs) and causality assessment in patients under Antepil® treatment.

ADRs	Causality		Total
	Possible	Probable	
Gastrointestinal disorders	2	1	3
Visual problems	3	5	8
Drowsiness	0	7	7
Dizziness	1	5	6
Dysarthria	1	2	3
Bleeding gums/hyperplasia	0	7	7
Memory loss	0	4	4
Walk instability	0	1	1
Tremor in hands	1	0	1
Headaches	0	1	1
Belly swelling	1	0	1
Irritability	0	1	1
Nervousness	0	1	1
Total	9	35	44

Table 5.
Detected adverse drug reactions (ADRs) and causality assessment in patients under Comitoína® treatment.

None of the patients developed skin reactions, an adverse effect observed in a clinical trial with healthy volunteers that was carried out by our research group [13]. It deserves to be mentioned that oral chronic administration of PHT induces microsomal EPHX, which could explain why the percentage of subjects with toxicity is higher at the early stage of the treatment (healthy volunteers) than after a chronic one (patients participating in the pharmacovigilance program). A lesser exposure of reactive metabolite during chronic administration could be due to enzyme induction by PHT.

Although the number of ADRs was greater under Comitoína® treatment, the severity of such reactions was less intense as no seizures due to high concentrations was carried out reported.

One limitation of the study was self-reported data of ADRs when patients filled the form. This can inherently bias the number and severity of the reported reactions.

For both brands, when seizures and low concentrations were present, lack of efficacy was suspected, and then, the dose was immediately increased in order to achieve therapeutic concentrations.

Mean dose and mean plasma and salivary concentration of PHT that were able to control the seizures for Antepil® and Comitoína are shown in **Tables 6** and **7,** respectively.

As the three main enzymes that participate in PHT metabolism are polymorphically expressed and the genetic variants are responsible for changes in the enzyme activity, our research group has also evaluated the effect that these polymorphisms have on PHT metabolism. The genotypic frequencies obtained for CYPs are in accordance with the ones reported for Caucasian population [34]. Thirty percentage of the patients were intermediate, and 2% were poor metabolizers for CYP2C9, whereas 20% were intermediate metabolizers for CYP2C19. No poor metabolizer was found for CYP2C19. Regarding EPHX, 44% of the patients had an intermediate, 10% an increased, and 46% a decreased enzyme activity. Although 46% of the patients had a decreased EPHX activity, none of the patients reported cutaneous reactions. As it was stated, not only the detoxification pathway but also the rate of formation of this toxic metabolite (arene oxide) must be taken into account. Both formulations behave as slow-release tablets. Moreover, during chronic administration, a lesser exposure of reactive metabolite can be experienced due to the enzyme induction PHT provokes. Our results also evidenced a predominant role of CYP2C9 in PHT biotransformation, while CYP2C19 seems to have a predominant role in p-HPPH biotransformation [31].

Dose (mg/kg)	[Po] (mg/L)	[So] (mg/L)
4.03 (3.77–4.29)	7.12 (5.81–8.44)	0.626 (0.491–0.760)

Table 6.
Mean (95% CI) PHT dose and mean plasma and salivary concentrations in patients with controlled seizures under Antepil® treatment.

Dose (mg/kg)	[Po] (mg/L)	[So] (mg/L)
4.34 (4.12–4.55)	9.14 (8.08–10.2)	0.930 (0.780–1.08)

Table 7.
Mean (95% CI) PHT dose and mean plasma and salivary concentrations in patients with controlled seizures under Comitoína® treatment.

A bioequivalence parallel design study in saliva was also carried out with these data. According to the results obtained in this study, for the three parameters under evaluation (Css, Cmax, and PTF), bioequivalence between Antepil® and Comitoína® can be concluded. This procedure of parallel assay, with replicate evaluation of drug exposure, becomes a valuable solution to demonstrate bioequivalence of such products [35].

5. Conclusions

Both oral formulation of PHT show a uniform behavior in the population studied. The dose increase caused a disproportionate increase in plasma concentrations (Michaelis-Menten kinetics), as referenced in the literature.

No statistically significant differences were detected between the normalized doses received by both sexes or in the salivary concentrations or plasma concentrations obtained with such doses.

The presence of secondary peaks in salivary curves revealed the recirculation processes already known for PHT.

Adverse drug reactions referenced by patients did not deserve medical intervention in most cases, except for the appearance of seizures with high PHT concentrations.

CYP2C9 polymorphisms affect mainly PHT concentrations, while CYP2C19 polymorphisms affect mainly p-HPPH concentrations, which verify the predominant role that CYP2C9 has in PHT metabolism and CYP2C19 in p-HPPH metabolism. A decreased EPHX activity did not evidence arene oxide accumulation as no cutaneous reactions were observed.

According to the results obtained in the parallel study, switchability between the two commercial brands can be inferred.

In summary, mean (95% CI) PHT dose of 4.34 (4.12–4.55) mg/kg of Comitoína® and 4.03 (3.77–4.29) mg/kg of Antepil® achieved effective and safe concentrations of PHT.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.80295

Author details

Marta Vázquez[1*], Pietro Fagiolino[1], Cecilia Maldonado[1], Natalia Guevara[1], Manuel Ibarra[1], Isabel Rega[2], Adriana Gómez[3], Antonella Carozzi[4] and Carlos Azambuja[4]

1 Pharmaceutical Sciences Department, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay

2 Neurology Clinics, Hospital Maciel, Montevideo, Uruguay

3 COSEM Clinics, Montevideo, Uruguay

4 Genia-Genetics Molecular Laboratory, Montevideo, Uruguay

*Address all correspondence to: mvazquez@fq.edu.uy

References

[1] Goldenberg MM. Overview of drugs used for epilepsy and seizures. Etiology, diagnosis, and treatment. Pharmacy and Therapeutics. 2010;**35**(7):392-415

[2] Yaari Y, Selzer ME, Pincus JH. Phenytoin: Mechanisms of its anticonvulsant action. Annals of Neurology. 1986;**20**(2):171-184

[3] Patsalos PN, Berry DJ, Bourgeois BF, Cloyd JC, Glauser TA, Johannessen SI, et al. Antiepileptic drugs—Best practice guidelines for therapeutic drug monitoring: A position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 2008;**49**(7):1239-1276

[4] Maldonado C, Fagiolino P, Vázquez M, et al. Therapeutic carbamazepine (CBZ) and valproic acid (VPA) monitoring in children using saliva as a biologic fluid. Journal of Epilepsy and Clinical Neurophysiology. 2008;**14**(2):55-58

[5] Ibarra M, Vázquez M, Fagiolino P, Mutilva F, Canale A. Total, unbound plasma and salivary phenytoin levels in critically ill patients. Journal of Epilepsy and Clinical Neurophysiology. 2010;**16**(2):69-73

[6] Fagiolino P, Vázquez M, Maldonado C, et al. Usefulness of salivary drug monitoring for detecting efflux transporter overexpression. Current Pharmaceutical Design. 2013;**19**(38):6767-6774

[7] Posti J. Saliva-plasma drug concentration ratios during absorption: Theoretical considerations and pharmacokinetic implications. Pharmaceutica Acta Helvetiae. 1982;**57**:83-92

[8] Vázquez M, Fagiolino P, De Nucci G, Parrillo S, Piñeyro A. Post-prandial reabsorption of paracetamol. European Journal of Drug Metabolism and Pharmacokinetics. 1993 (Special Issue: 177-183. Proceedings of the 5th. Eur. Cong. Biopharm. Pharmacokinet., Brussel (Belgium), 1993)

[9] Vázquez M, Fagiolino P, Lorier M, Guevara N, Maldonado C, Ibarra M, Montes MJ, Retamoso I. Secondary-peak profile of methadone in saliva after administration of multiple doses in patients with chronic pain. Current Topics in Pharmacology. 2015;**19**:21-26

[10] Wu MF, Lim WH. Phenytoin: a guide to therapeutic drug monitoring. Proceedings of Singapore Healthcare. 2013;**22**:198-202

[11] Richens A. Clinical pharmacokinetics of phenytoin. Clinical Pharmacokinetics. 1979;**4**(3):153-169

[12] Thorn CF, Whirl-Carrillo M, Leeder JS, Klein TE, Altman RB. PharmGKB summary: Phenytoin pathway. Pharmacogenetics and Genomics. 2012;**22**:466-470

[13] Vázquez M, Fagiolino P, Alvariza S, Ibarra M, Maldonado C, Gonzalez R, Laborde A, Uria M, Carozzi A, Azambuja C. Skin reactions associated to phenytoin administration: Multifactorial cause. Clinical Pharmacology and Biopharmaceutics. 2014;**3**:125. doi: 10.4172/2167-065X.1000125

[14] Wormhoudt LW, Commandeur JN, Vermeulen NP. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: Relevance to xenobiotic metabolism and toxicity. Critical Reviews in Toxicology. 1999;**29**:59-124

[15] Ingelman-Sundberg M, Gaedigk A, Brockmöller J, Goldstein JA, Gonzalez

FJ, et al. The Human Cytochrome P450 (CYP) Allele Nomenclature Database. Available from: http://www.cypalleles.ki.se [Accessed: May 30, 2018]

[16] Pinarbasi H, Silig Y, Pinarbasi E. Microsomal epoxide hydrolase polymorphisms. Molecular Medicine Reports. 2010;**3**:723-727

[17] Mauro LS, Mauro VF, Brown DL, Somani P. Enhancement of phenytoin elimination by multiple-dose activated charcoal. Annals of Emergency Medicine. 1987;**16**:1132-1135

[18] Howard CE, Roberts RS, Ely DS, Moye RA. Use of multiple-dose activated charcoal in phenytoin toxicity. The Annals of Pharmacotherapy. 1994;**28**:201-203

[19] Glick TH, Workman TP, Graufberg SV. Preventing phenytoin intoxication: Safer use of a familiar anticonvulsant. The Journal of Family Practice. 2004;**53**:197-202

[20] Fagiolino P, Vázquez M, Eiraldi R, Maldonado C, Scaramelli A. Influence of efflux transporters on drug metabolism. Theoretical approach for bioavailability and clearance prediction. Clinical Pharmacokinetics. 2011;**50**:75-80

[21] Alvariza S, Fagiolino P, Vázquez M, Feria-Romero I, Orozco-Suárez S. Chronic administration of phenytoin induces efflux transporter overexpression in rats. Pharmacological Reports. 2014;**66**:946-951

[22] Alvariza S, Ibarra M, Vázquez M, Fagiolino P. Different phenytoin oral administration regimens could modify its chronic exposure and its saliva/plasma concentration ratio. Journal of Medical and Pharmaceutical Innovation. 2014;**1**:35-43

[23] Vázquez M, Fagiolino P, Mariño E. Concentration-dependent mechanisms of adverse drug reactions in epilepsy. Current Pharmaceutical Design. 2013;**19**:6802-6808

[24] Prasad K, Al-Roomi K, Krishnan PR, Sequeira R. Anticonvulsant therapy for status epilepticus. Cochrane Database of Systematic Reviews. 2005;**4**:CD003723. DOI: 10.1002/14651858.CD003723.pub2

[25] Browne TR, Kugler AR, Eldon MA. Pharmacology and pharmacokinetics of fosphenytoin. Neurology. 1996;**46**:S3-S7

[26] Jamerson BD, Dukes GE, Brouwer KL, Donn KH, Messenheimer JA, Powell JR. Venous irritation related to intravenous administration of phenytoin versus fosphenytoin. Pharmacotherapy. 1994;**14**:47-52

[27] Uribe-San-Martín R, Ciampi E, Uslar W, Villagra S, Plaza J, et al. Risk factors of early adverse drug reactions with phenytoin: A prospective inpatient cohort. Epilepsy & Behavior. 2017;**76**:139-144

[28] Brodie MJ, Mintzer S, Pack AM, Gidal BE, Vecht CJ, Schmidt D. Enzyme induction with antiepileptic drugs: Cause for concern? Epilepsia. 2013;**54**:11-27

[29] Chung WH, Wang CW, Dao RL. Severe cutaneous adverse drug reactions. The Journal of Dermatology. 2016;**43**:758-766

[30] Savio E, Fagiolino P, Solana G, Parente E, León A. Development of water/oil emulsion. Bioavailability in rats. STP Pharma Sciences. 1991;**1**:379-385

[31] Guevara N, Maldonado C, Uría M, González R, Ibarra M, et al. Role of CYP2C9, CYP2C19 and EPHX polymorphism in the pharmacokinetic of phenytoin: A study on Uruguayan Caucasian subjects. Pharmaceuticals. 2017;**10**:73. DOI: 10.3390/ph10030073

[32] Vázquez M, Fagiolino P, Maldonado C, et al. Hyperammonemia associated with valproic acid concentrations. BioMed Research International. 2014;**2014**:217269. DOI: 10.1155/2014/217269

[33] Maldonado C, Guevara N, Queijo C, et al. Carnitine and/or acetylcarnitine deficiency as a cause of higher levels of ammonia. BioMed Research International. 2016;**2016**:2920108. DOI: 10.1155/2016/2920108

[34] Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9polymorphisms: A comprehensive review of the *in-vitro* and human data. Pharmacogenetics. 2002;**12**:251-263

[35] Guevara N, Fagiolino P, Vázquez M, Maldonado C. Replicate evaluation of drug exposure to study bioequivalence between two brands of phenytoin in patients. Current Topics in Pharmacology. 2018;**22** (in press)