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Research Article

Different Phenytoin Oral Administration Regimens Could Modify Its Chronic Exposure and Its Saliva/Plasma Concentration Ratio

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ABSTRACT

The aim of the present study was to compare plasma and saliva pharmacokinetic data obtained in healthy subjects after two different phenytoin administration regimens, having the same administration rate but different dosing interval, in order to investigate if efflux transporter induction at the blood brain barrier could be minimized, and hence a new approach for avoiding refractory epilepsy might be proposed. Six from twelve volunteers, to whom 600 mg every 72 h or 100 mg every 12 h of phenytoin were administered during 10 days, completed a two-way, two-period crossover study. Two fractions of stimulated saliva were collected simultaneously with plasma samples throughout a scheduled 24-hour period on day 10, and during the subsequent 4-day washout period.

Average plasma (P) concentrations of both the parent drug ($p<0.01$) and the main metabolite (p-hydroxyphenylphenytoin, $p<0.05$) showed higher values when 600 mg each 72 h were given. Also, the saliva first-fraction (S1) average concentration was higher ($p<0.05$) after 600 mg every 72 h. Conversely, S1/P average concentration ratio was higher ($p<0.01$) after 100 mg every 12 h. Half-lives, metabolic ratios, saliva second-fraction (S2) average concentrations, and S1/S2 ratios of average levels were not significantly different between treatments.

Results reveal that, even though the input rate was kept constant, higher doses given at long dosing intervals displayed higher systemic exposures and lower saliva/plasma exposure ratio than lower doses given at short intervals . Our hypothesis, supported by studies carried out with rats and theoretical approaches, is that efflux transporter overexpression at hepatocytes and at salivary cells would be induced by the higher local phenytoin exposures provoked by 600 mg every 72 h and by the persistent systemic exposures provoked by 100 mg every 12 h, respectively. The maintenance of systemic phenytoin concentration would be foreseen as one of the causes leading to antiepileptic treatment refractoriness, since a higher brain-to-plasma transportation of drug could be induced.

Maybe once-daily dosing of phenytoin would be preferable to twice or three times a day administration regimens. Despite the fact that avoiding peak-trough oscillation was always the aim of pharmacotherapy, it might be more appropriate for patients under phenytoin treatments to increase both dose and dosing interval in order not to contribute to the installation of pharmacoresistance.

Keywords: Phenytoin, dosage regimens, efflux transporters, saliva/plasma ratio, refractoriness

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INTRODUCTION:

Seventy to eighty per cent of epileptic patients are successfully treated with the commercially available antiepileptic drugs (AEDs). However, there are still 20-30 % of individuals with intractable or uncontrolled seizures (1), even at maximum tolerated doses of AEDs.

Refractory epilepsy has been explained by different mechanisms, ones involving molecular alterations in several neurotransmitter receptors, and others, involving over-expression of multidrug transporters proteins (ATP-binding cassette superfamily, ABC) at the blood brain barrier (BBB), resulting the former in partial or total drug ineffectiveness and impairment of drug penetration in the brain the latter (2-5). The hypothesis of over-expression of efflux transporters has been the most studied to explain this refractoriness so far. Lack of efficacy of AEDs which are substrates of these transporters would be the consequence of limited entrance of the therapeutic agent in the brain.

High frequency of seizures before the start of treatment is also associated with refractoriness (6, 7) as these uncontrolled seizures can induce over-expression of drug transporters at different parts of the central nervous system (CNS) such as the epileptogenic focus, the BBB, and even at distant areas of the body (8, 9). There is evidence indicating high expression of members of this family such as P-glycoprotein (Pgp) and multi-drug resistance proteins (MRPs) at the BBB or glial cells or neurons of nonresponsive patients (10, 11).

Evidence of Pgp over-expression in brain tissue in several animal models of epilepsy was found (12). Uncontrolled seizures can be the cause of this up regulation, but some AEDs themselves can cause efflux transporter over-expression leading to treatment failure and thus refractoriness (13, 14).

Many efforts have been made to change this situation: 1) development of drugs non substrates of these transporters; 2) use of efflux transporters inhibitors; 3) use of nanocarriers in order to avoid the active transport; among others. In the clinical setting this approach of overcoming transporters resistance by the use of inhibitors perhaps is not a good solution as these proteins are implied in dynamic efflux not only of drugs but also of endogenous compounds and toxins, and bearing in mind the chronic nature of epilepsy a permanent disruption of transporters by blocking them is likely to result in severe side effects.

Phenytoin (PHT) is a first-line anticonvulsant agent used in different types of epilepsy and the inductive capacity of this drug over Pgp and MRP2 expression in brain has already been reported (13). Our investigations in rats (15) confirmed those findings not only in brain but in different tissues and with different intensity: enterocyte, salivary glands, hepatocytes, and finally in BBB, in decreasing order. Increasing doses were tested and a threshold inductive concentration was observed. Interestingly, one to seven days after drug interruption, depending of the administered dose, basal expression of transporters recovered, giving evidence that induction was local concentration and time dependent (16).

However, it has to be taken into account that the inductive effect of PHT not only impact on transporter expression in the brain but also in the rest of the body and this would directly condition the amount of drug available for the CNS. Over-expression of transporters can affect both bioavailability and clearance of the drug. PHT is cleared predominantly by hepatic route through CYP2C9 and CYP2C19. The so-mentioned non-linear kinetics of PHT by progressive clearance reduction was explained by our group (17) by means of deviating PHT from a region (hepatocytes) with high content of CYP2C9/CYP2C19 to a region (enterocytes) with low content of these enzymes due to the self-induction of the efflux transporter expression in the biliary canaliculi (15). These molecules secreted to the digestive tract can be reabsorbed, and hence, their elimination reduced.

The administration of a well-known blocker such as Verapamil (VPM) increased brain concentrations of PHT after cerebral perfusion in rats by cerebral inhibition of transporters (18). Given by oral route VPM also increased oral PHT bioavailability (19) by blocking intestinal efflux transporters. Our investigations in rats (20) suggested that VPM could have different impact on PHT kinetics regarding the time when the blocking is added to a five-day chronic intraperitoneal once-daily administration of PHT: 1) a decreased clearance (and/or increased bioavailability) after the second dose, when both efflux carriers and enzymes had not been induced yet; or 2) an increased clearance (and/or decreased bioavailability) after the fifth dose

of PHT, when efflux carriers and enzymes (mainly at the hepatocyte) had already been induced. Thus, efflux transporter could explain both the non-linear PHT pharmacokinetics and the interaction with VPM throughout chronic administration of the anticonvulsant. Hence, this fact would position efflux transporters not only as the main responsible for the lack of anticonvulsant response of drugs but also for their residence times in the body. Then, efflux transporters become the cornerstone of drug fate and indirectly of drug action.

Efflux transporters are present either at acinar or ductal cells (21) of salivary gland. Saliva was used by our research group in many investigations in order to detect efflux transporter over-expression (22, 23). When saliva is first stimulated and collected, drug concentration is the result of filtration through acini and thereafter the multiple subsequent exchanges between the luminal space and the blood contained in the capillaries surrounding ductal cells. If immediately after, a second stimulated saliva collection is carried out the equilibrium at the ductal cells is not restored, now the saliva drug concentration will resemble the free drug flowing throughout the arteries which irrigate the acini. In order to enhance the understanding of drug pharmacokinetics, a protocol that collects saliva in two fractions was recommended (23): fraction 1) which would correspond to drug concentration in the salivary gland (lower part of the ducts) once the first stimulation is completed (S1); and fraction 2) which would correspond to the upper part of the ducts after continuous stimulation (S2). A close relation could be envisaged between the upper saliva composition and the artery content, and between the lower saliva fluid and the interstitial content drained to the veins. Vein and artery concentrations could be surrogated by S1 and S2 saliva concentrations respectively. Thus, saliva S1 / free venous plasma concentration ratio could measure the extent of tissue/plasma distribution, and the S1/S2 saliva concentration ratio could measure the dilution or concentration of a drug in plasma after its circulation throughout the circulatory system. In other words, venous/artery (V/A) drug concentration ratio could be surrogated by the S1/S2 ratio (23).

The influence that instantaneous bioavailability and clearance has on the systemic exposure of a drug has been published recently (24). This could be assessed by the V/A, or S1/S2, ratio measured at any time. The higher or lower V/A is, the lower or higher drug absorption extent in relation with its elimination operates, respectively.

The aim of the present study was to compare plasma and saliva pharmacokinetic data obtained after two different PHT administration regimens with the same administration rate but with different dosing interval, in order to investigate if PHT efflux transporter induction at the BBB could be minimized, and hence a new approach for avoiding refractory epilepsy might be proposed.

MATERIALS AND METHODS:

Subjects and study design

Twelve healthy Caucasian subjects (6 men and 6 women) were recruited in an open-label, randomized, multiple-dose, two-period, crossover pharmacokinetic study of PHT. The study involved two different treatments having the same average input rate but with different dosing interval: 600 mg of PHT every 3 days, during 10 days (treatment A), and 100 mg of PHT every 12 hours during 10 days (treatment B). Dose of 600 mg was given as 200 mg every 2 h in order to minimize gastrointestinal fluid saturation of drug. A wash-out period of 5 days was kept between both treatments. Two brands of PHT were used: prompt-release Epanutin® capsules (Pfizer Laboratories) for six volunteers and slower-release tablets commercially available in Uruguay (Comitoina®, Roemmers Laboratories) for the other six.

The study protocol and informed consent form were designed according to the ethical guidelines for human clinical research and were approved by the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay). Written informed consent was obtained from all subjects before their entry in the study. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation, situated in ‘‘Dr. Juan J. Crottogini’’ Hospital (Montevideo, Uruguay).

Volunteers came to the Centre the first day of each period, with an eight-hour overnight fasting period. Subjects received breakfast at 7:30 am, and PHT was administered at 8:00. Standardized meals (lunch, tea, dinner and breakfast) were provided at four, eight, twelve and twenty-four hours after dose administration. After that, the volunteers were released from the Centre up to day 10. On the

tenth day of each period, subjects repeated the same schedule.

Sample collection

On day 10, blood samples were withdrawn from the antecubital vein through cannulation and placed into heparinized tubes prior to dosing (0 hour), and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 24, 36, 48, 72, and 96 hours after dose. Plasma was separated by centrifugation and stored at -25 °C until analysis.

Saliva samples were obtained simultaneously with blood. S1 was collected by placing 50 mg of citric acid on the tongue in order to stimulate salivation and saliva was spit into tubes up to 1 mL of volume. Intermediate salivations were discarded and the procedure was repeated to obtain the second fraction S2. Samples were stored at -25 °C until analysis.

Analytical method for drug determination

PHT and para-hydroxyphenyl-phenytoin (pHPPH) plasma concentrations and PHT saliva concentrations were determined by a high performance liquid chromatography (HPLC) method based on a procedure previously developed (25) with minor modifications.

Fifty microliters of internal standard solution (nitrazepam, 16 µg/mL in methanol) were added to 500 µL of plasma or saliva. The extraction of analytes was performed by adding 3 mL of ethyl acetate and then vortexed for 1 minute. After centrifugation, the supernatant was separated and dried under nitrogen stream at 37-40°C. Dry residue was dissolved with 100 µL of mobile phase and 20 µL injected into a Dionex Ultimate 3000 series chromatograph. A Phenomenex®Luna C18 (5µm, 150 mm x 4.6 mm) column was used as a reversed stationary phase. The mobile phase was a mixture of water/methanol/acetonitrile (43:47:10) pumped with a flow rate of 1.0 mL/min. The column compartment was kept at 40°C and the wavelength detection was 220 nm. Under these conditions, the retention times of analytes were 3.5, 4.5 and 5.8 for p-HPPH, PHT and nitrazepam respectively.

The HPLC method was linear between 0.5 (the lower limit of quantification, LLOQ) and 25.0 mg/L for PHT in plasma, between 0.05 (LLOQ) and 3.0 mg/L for p-HPPH in plasma and between 0.2 (LLOQ) and 7.0 mg/L for PHT in saliva. Inter and intraday coefficients of variation (CVs) were below 15% and the accuracy of the method was between 85-115% for both analytes.

Pharmacokinetic parameters and their meaning

The area under the steady-state PHT and pHPPH plasma or PHT saliva concentration versus time curves ($AUC_{ss\ 0\text{-}T}$) were calculated using the trapezoidal rule until 72 hours for Treatment A and 24 hours for Treatment B. Beta (β), the first order elimination rate constant, was calculated from the slope of the terminal log-linear concentration-time regression in Treatment A. In Treatment B, β was estimated once the drug was discontinued (from day 11^{th}). Steady state average PHT and p-HPPH plasma or PHT saliva concentrations ($[PHT]_{ss}$, $[pHPPH]_{ss}$, $S1_{ss}$, $S2_{ss}$) were determined as $AUC_{ss\ 0\text{-}T}$ /T, being T: 72 h for treatment A and 24 h for treatment B. Elimination half- life ($t_{1/2}$) was calculated as 0.693/β just for PHT.

pHPPH/PHT plasma concentration ratio was calculated from their respective steady state average concentrations. Also, S1-saliva/plasma PHT concentration ratio (S1/P) and S1/S2 PHT saliva concentration ratio were calculated using steady state average concentrations.

Metabolic ratio (pHPPH/PHT) refers to the actual status of bioavailability and clearance means of both parent drug and the metabolite involved in the main route of PHT biotransformation. S1/S2 ratio informs about the prevalence of absorptive events throughout the monitored interval. The lower it is, the more frequent PHT inputs during the monitored time. A graph showing S1 and S2 versus time will inform about the precise time PHT absorption occurs (S2 higher than S1). S1/P ratio more accurately states the salivary gland / plasma ratio since S1 and P are closely involved in the equilibrium between saliva and plasma at the salivary ducts. Any change between treatments should be viewed opposed to what is happening at the BBB between brain and plasma, since efflux transporters extrude the drug to the saliva and not to the blood. If an increase in S1/P is obtained an unidirectional transport towards the saliva will be the cause, provided not changes in plasma protein binding were carried out, and then, the efflux transporter will be likely involved since they are located at the apical membrane of cells surrounding the salivary ducts.

Statistical Analysis

Mean and standard deviation were calculated for all pharmacokinetic parameters. Paired t-Student test was used for comparing mean

values. Significances were assessed when type I error was less than five percent ($p<0.05$). Ninety-five percent confidence intervals (95%CI) were constructed when appropriate.

RESULTS

Only 6 volunteers (5 male and 1 female) completed the study. The other 6 were withdrawn because of cutaneous adverse reaction (rash) occurrence. This high rate of adverse reaction to PHT was analyzed and explained elsewhere (26). Four of these individuals who experienced rash were relatives. Except for 1 female, who was taking estrogens as oral contraceptive, all the other excluded individuals showed, as relevant characteristics, shorter half-lives, lower activity of their epoxide hydrolase, and higher plasma level of p-HPPH. Intermediate arene oxide accumulation, produced during the hydroxylation of PHT, might be the cause. Rash is not uncommon when PHT is introduced into the therapy, and treatment continuation overrides this secondary effect in most of the cases. Since this study was not designed to investigate adverse reactions to PHT, subjects with rash were excluded from the analysis. Once PHT was discontinued the symptoms disappeared.

It is important to refer (26) that cutaneous adverse reaction were mostly observed by the end of treatment B, and so, the higher dose administered (600 mg, under treatment A) could not be the cause of such events. Besides, no difference in adverse effects between brands were observed. Apart from the above mentioned pharmacokinetic characteristics the sex of individuals seemed to be relevant, being women more susceptible to experience rash.

Table 1 summarizes the anthropometric characteristics of subjects who completed the two treatments assayed without secondary effects.

Subject	Sex	Race	Age	Weight	Height	BMI	Smoker	Formulation
			years	kg	cm	kg/m2		
E1-M	male	caucasian	19	76	174	25	no	Epanutn
E3-M	male	caucasian	23	87	173	29	no	Epanutn
C1-M	male	caucasian	26	84	180	26	yes	Comitoina
C2-M	male	caucasian	34	64	170	22	yes	Comitoina
C3-M	male	caucasian	22	86	180	26	no	Comitoina
C6-F	female	caucasian	26	68	164	26	yes	Comitoina

Table 1: Subject data

Two men completed the study with Epanutin® and 3 men and 1 woman with Comitoina®.

Table 2 shows mean (± standard deviation) pharmacokinetic parameters obtained after treatments A and B. Table 3 summarizes the A/B treatment ratio for each parameter in each volunteer, and the respective mean (± standard deviation). Figure 1 displays each mean of the A/B pharmacokinetic parameter ratio with the respective 95%CI. Figures 2 and 3 show plasma and saliva mean concentration-time curves of PHT throughout time after doses given on day 10, respectively.

Treatment A: 600 mg of PHT every 72 h								
	[PHT]ss mg/L	**[pHPPH]ss mg/L**	**S1ss mg/L**	**S2ss mg/L**	**S1/S2**	**S1/P**	**t1/2 h**	**pHPPH / PHT**
mean	3.85	0.0815	0.338	0.371	0.908	0.089	20.54	0.0272
SD	2.77	0.0295	0.223	0.230	0.113	0.012	11.44	0.0146
95%CI	1.92	0.0204	0.155	0.159	0.078	0.009	7.93	0.0101
Treatment B:	100 mg of PHT every 12 h							
	[PHT]ss mg/L	[pHPPH]ss mg/L	S1ss mg/L	S2ss mg/L	S1/S2	S1/P	t1/2 h	pHPPH / PHT
mean	2.81	0.0762	0.294	0.308	0.960	0.111	16.02	0.0340
S.D.	1.99	0.0326	0.172	0.178	0.096	0.019	8.95	0.0169
95%CI	1.30	0.0213	0.112	0.116	0.063	0.013	5.85	0.0110

Table 2: Mean (n=6) and standard deviation (SD) of pharmacokinetic parameters after 10 days of chronic oral administration of phenytoin (PHT)

As it can be observed in table 3, $[PHT]_{ss}$, $[pHPPH]_{ss}$, S1, and S1/P showed significant differences between treatments, since the value 1 was not included within the 95%CI of their B/A treatment ratios. Higher mean PHT plasma exposure in veins observed under

treatment A ($p<0.01$) was accompanied by similar behavior of its main metabolite pHPPH ($p<0.05$). So, similar increases in bioavailability or decreases in clearance accounted for both the parent drug and metabolite when PHT was administered in a higher dose (600 mg) in a longer interval of time (every 72 h). Also a higher mean PHT exposure in the first fraction (S1) of saliva ($p<0.05$) was observed under treatment A.

Subject	[PHT]ss mg/L	[pHPPH]ss mg/L	S1ss mg/L	S2ss mg/L	S1/S2	S1/P	t1/2 h	pHPPH / PHT
E1-M	1.412	0.997	1.233	1.255	0.983	0.873	1.229	0.706
E3-M	1.580	1.774	1.058	0.930	1.138	0.670	1.483	1.123
C1-M	1.371	1.592	1.285	1.780	0.722	0.937	0.531	1.161
C2-M	1.228	0.956	0.991	0.905	1.096	0.807	1.227	0.779
C3-M	1.694	1.171	1.234	1.474	0.837	0.729	1.150	0.692
C6-F	1.214	1.264	0.992	1.102	0.900	0.817	1.106	1.041
mean	1.416	1.292	1.132	1.241	0.946	0.806	1.121	0.917
S.D.	0.191	0.328	0.134	0.339	0.158	0.096	0.317	0.215
95% CI	0.153	0.262	0.107	0.271	0.126	0.077	0.254	0.172

Table 3: Treatment A / treatment B pharmacokinetic parameters ratio in each individual

Conversely, tissue (salivary gland) / plasma ratio, measured as the ratio between drug concentrations flowing throughout salivary ducts (S1) and throughout veins (P), was higher ($p<0.01$) after giving lower doses of PHT (100 mg) in shorter intervals of time (every 12 h). So, a higher transference from plasma to saliva could be inferred after treatment B.

Ratio between PHT saliva concentrations (S1/S2) did not suffer any changes regarding the frequency of dose administration. Half-lives, estimated from 24 h (treatment A) or 12 h (treatment B) after drug discontinuation, did not show differences between treatments.

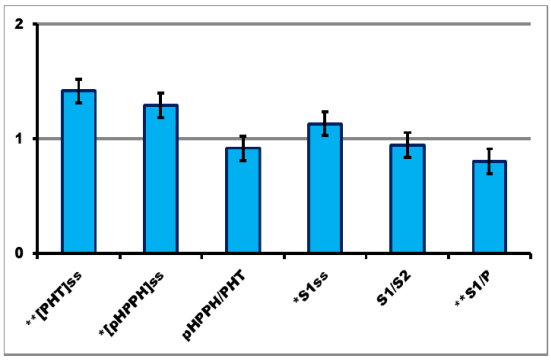

Figure 1: Mean (± 95%CI) of treatment A / treatment B pharmacokinetic parameter ratios
* ($p<0.05$) and ** ($p<0.01$) significant difference from 1

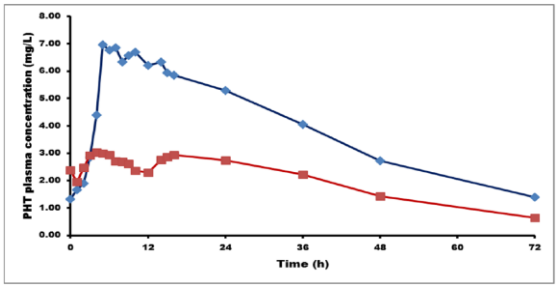

Figure 2: Mean phenytoin (PHT) plasma concentration – time curves in six healthy volunteers, after 10 days of 600 mg every 72 h (blue line) or 100 mg every 12 h (red line) administrations

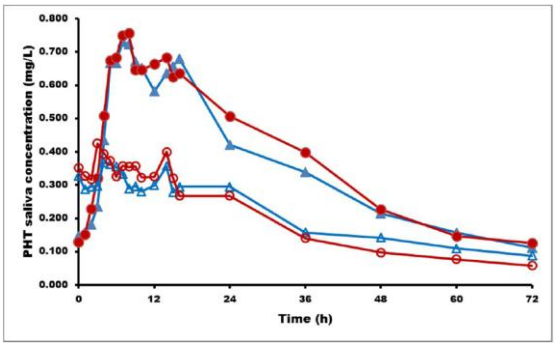

Figure 3: Mean phenytoin (PHT) saliva concentration – time curves in six healthy volunteers after 10 days of 600 mg every 72 h (solid symbols) or 100 mg every 12 h (open symbols) administrations. First (S1) and second (S2) fractions of collected saliva are represented by blue and red lines, respectively

DISCUSSION

Higher PHT plasma exposure after 600 mg every 72 h could be explained by means of a decreased average clearance. When Michaelis-Menten elimination kinetics operates, high doses administered with spaced intervals of time normally yield higher steady state average concentrations than low doses administered more frequently. The most frequent way of drug administration is by means of intravenous perfusion at constant

rate, which renders steady state concentration (Css) values without any oscillation. If an intermittent intravenous bolus administration pursuits the treatment, with the same input rate (dose-by-interval), the concentration will be above Css most of the time. This is because of the negative concavity shape that the concentration-time curve displays. Hence, elimination is lower in comparison to the input, and then, a higher Css will be attained, in agreement with a lower average clearance.

Our recent hypothesis of efflux transporter overexpression at the splanchnic region (mainly at the biliary canaliculi) is in agreement with the Michaelis-Menten equation, but the less efficient work of hepatic enzymes would not be consequence of their saturation but of the deviation of molecules to a site with a lower capacity to metabolize PHT: the intestine. Once the drug is in the intestine it could be reabsorbed, and hence, its hepatic metabolism could be bypassed.

According to previous reports (27, 24) artery concentrations are higher than the venous ones during drug absorption, and so, for non-ionized lipophilic molecules S2 must be higher than S1. Lower S1/S2 salivary concentration ratio during drug absorption might be expected.

Our results of S1/S2 ratio showed similar absorption-elimination balance within the dose interval between treatments A and B. One hundred milligram administered every 12 h meant two doses given during a 24-h interval, period used to calculate both S1 and S2 average drug concentration. Thus, several samples were taken while exogenous PHT was entering the body, then absorption predominated over the elimination and so, S1/S2 ratio should have been decreased because of this phenomenon. Conversely, with treatment A, after a 12-hour period no more exogenous PHT could be entering the body. Hence, several samples within the decay period of S1 and S2 curves were used to estimate S1/S2 salivary average concentration ratio. Despite this, S1/S2 ratio for treatment A was not higher than that for treatment B. Then, reentrances of systemic PHT under treatment A should be the cause for observing this lower than predicted S1/S2 ratio.

As it can be seen in figure 3, treatment A shows inverted S1/S2 ratio in comparison to treatment B. So, during a period in which no more entry of exogenous PHT is operating, this lower S1/S2 (or even inverted) ratio reveals a higher plasma-intestine-plasma recirculation of PHT. This process of recirculation seemed to operate by pulses, probably activated by food intake, since a sudden inversion of S1/S2 happened, otherwise S1/S2 ratio would be decreased, but always with a value above 1 (24).

Higher local concentrations after 600 mg doses could induce higher expression of transporters in comparison to the lower concentration obtained after a 100 mg PHT dose. Twenty four to 48 hours later the effect of extruding the drug from the hepatocyte to the intestine seemed to experience its maximal intensity, afterwards it decayed progressively throughout time once local concentration became insufficient to maintain this inductive activity. To our knowledge this could be the first evidence, not just confirming the suspicion of PHT reabsorption (28-31), but supporting the concentration-dependent induction of efflux transporter as a mechanism for the PHT self-decreasing hepatic clearance under its chronic administration.

The metabolite p-HPPH seemed to follow similar behavior to the parent drug. Maybe it has the same mechanism of transport from the liver to the intestine, and its hepatic clearance would also be reduced after 600 mg of PHT every 72 hours. There are no studies in the literature evidencing p-HPPH as an efflux transporter substrate, however, and considering the structural similarities between PHT and p-HPPH, it seems to be feasible. Under this assumption, no changes in the bioavailability of metabolite should be foreseen.

Interestingly, a higher S1/P value was found under treatment B. PHT average plasma and saliva (S1) concentrations were lower than under treatment A. Regarding the lower plasma concentration observed under treatment B, no displacement of PHT plasma protein binding should have taken place. The higher S1/P ratio should be then understood as a more effective distribution from blood to the salivary gland because of maintained inductive PHT concentrations throughout time. The opposite should be envisaged for the equilibrium between blood and brain since efflux carriers transport the drug from the brain to the intravascular space.

The lack of inductive PHT effect at the salivary glands under treatment A is not surprising since a high peak-trough oscillation was obtained, and so, non inductive systemic

concentration of the drug could be kept throughout the dosing interval. The opposite could be the case regarding intestinal and hepatic PHT levels, since drug was under continuous recirculation because of the higher inductive effect caused by the 600 mg dose given at the beginning of the dose interval. The typical shape for the decay of PHT plasma concentration, accordingly with Michaelis-Menten elimination kinetics such as it has been reported in the literature (32, 33), could now be more precisely assigned to several plasma-gastrointestinal cycles which are losing their intensities because of a gradual decrease in the inductive status of efflux transporters at the hepatocyte. This phenomenon runs in parallel with the concept of an increasing in clearance meanwhile PHT plasma concentrations are decreasing throughout time.

It results interesting the progressive loss of inductive power that treatment A would have on the BBB by the end of the dosing interval due to systemic PHT concentration decay. Consequently, when the next dose is given the antiepileptic might penetrate the brain as effectively as it did under the previous dose. This would not be the case under treatment B since the maintenance of continuous PHT levels reduces its entrance to the brain. Any dose increase with the same dose interval would increase its systemic level, worsening drug access to the biophase.

Maybe once-daily dosing of PHT would be preferable to twice or three times a day administration regimens. Despite the fact that avoiding peak-trough oscillation was always the aim of pharmacotherapy, it might be more appropriate for patients under PHT treatments to increase both dose and dosing interval in order not to contribute to the installation of pharmacoresistant epilepsy (34).

CONCLUSION

PHT given chronically produces an inductive effect on the expression of efflux transporters which depends on the dose and the dosing interval. Higher local tissue concentration yields higher inductive effect. Once PHT disappears from the tissue, the efflux transporter expression returns to basal level.

The higher the dose was (600 mg), the higher the transporter expressed. This higher expression at the hepatocyte caused a lower clearance of PHT, and consequently higher systemic exposure was obtained after treatment A. Because of the longer dosing interval (72 h) the increased expression of transporter at the salivary gland was not maintained by the end of the period and transporters recovered their basal expression.

Low doses of 100 mg administered every 12 h were not enough to induce efflux transporter in the hepatocyte, and thereafter to decrease PHT clearance to the extent of a 600 mg dose. However, a sustained PHT systemic exposure was able to induce plasma-to-saliva transportation of drug. Similarly, a higher brain-to-plasma transportation of PHT would be envisaged.

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