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# Verapamil effect on phenytoin pharmacokinetics in rats

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Interaction on efflux transporter

**Summary** Efflux transporter and enzyme overexpression can be induced by certain antiepileptic drugs. Phenytoin (PHT) is at the same time substrate and inducer of CYP2C isoenzymes and efflux carriers. Its inductive effect has been postulated to be concentration and time-dependent. Since verapamil (VPM) is a well known substrate and inhibitor of P-glycoprotein, its administration could modify PHT systemic exposure. The objective of this work was to determine if single doses (40 mg/kg) of VPM might change PHT body fate in the same way when given at the beginning or several days after 100 mg/kg of PHT daily doses were started. Both drugs were administered intraperitoneally to female Sprague Dawley rats. VPM increased plasma PHT concentrations after one day of treatment, while a decrease in PHT plasma exposure was observed when VPM was added at the fifth day of the antiepileptic treatment.

These results suggested that VPM would have different impact on PHT pharmacokinetics, depending on the level of expression of both efflux transporters and enzymes. Before the hepatic cells could acquire a high content of enzymes due to the inductive effect of PHT dosing, VPM decreased the predominant intestinal clearance of PHT. But, once the enzymatic machinery at the hepatocyte became more important than that at the intestine, although ineffective because of the high hepatobiliary efflux transporter overexpression, VPM blockade from the liver resulted in an increased total PHT clearance.
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## Introduction

Phenytoin (PHT) is an antiepileptic drug which could be transported through the cell membranes by means of efflux carriers (Lu et al., 2004; Miller et al., 2008; Van Vliet et al.,

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2006). It was reported that PHT pharmacoresistance might be due to this mechanism of transport at the blood—brain barrier (Lazarowski et al., 2007). Both efflux transporters and cythocrome P450 enzymes would play significant roles in the bioavailability and the concentration-dependent clearance of PHT (Fagiolino et al., 2011). A study carried out by our group (Alvariza et al., 2013) demonstrated that chronic oral administration of PHT induced Pgp expression at different tissues in a time-concentration dependent way, showing the highest intensity at the intestinal and hepatobiliary sites. Also, Pgp overexpression was detected in the brain after chronic exposure of antiepileptic inducer drugs in rats (Wen et al., 2008).

It has been shown that the administration of Verapamil (VMP), a well known blocker of P-glycoprotein (Pgp), was able to increase both brain concentrations of PTH when administered by intracerebral perfusion in rats (Potschka and Löscher, 2001), and oral bioavailability of PHT (Neerati et al., 2011) when administered by oral route, because of cerebral and intestinal efflux transporter inhibition, respectively. This study was carried out with single oral doses of both VPM and PHT. Due to the relevant intestinal expression of CYP2C6 and CYP2C11 in rats (Lindell et al., 2003), oral bioavailability of PHT is not complete (Alvariza et al., 2013).

The main goal of the present work was to study whether a single intraperitoneal dose of VPM could interact with chronic intraperitoneal administration of PHT, in the same way as it was shown after oral coadministration of both drugs.

## Materials and methods

### Animals

Two groups (A and B) of six healthy female Sprague Dawley rats each, weighing 250—300 g, were housed in contiguous cages and fed at libitum. A twelve-hour light-dark cycle and an ambient temperature of 25 °C were maintained, before and during the experiments. Procedures involving animal care were conducted in agreement with the Official Mexican Norms (NOM-062-ZOO-1999) and the Ethical Committee of the Center of Research and Advanced Studies (project 512-12) and National Commission Scientific Research-IMSS, in Mexico.

### Treatments

Group A was daily intraperitoneal administered with 0.5 mL of vehicle solution for three days. On day 4, an intraperitoneal dose of 100 mg/kg of PHT (EPAMIN™ SP, solution for injection Pfizer Laboratories) was given. Before and after the dose, blood samples (150 μL) were withdrawn from the caudal vein and collected in tubes containing EDTA as anticoagulant (Minivacutainer™), every 20 min up to 3 h and then hourly up to 6 h. On day 5, an intraperitoneal dose of 40 mg/kg of VPM was administered 1 h before the PHT administration and blood samples were collected following the same schedule of the fourth day.

Group B was intraperitoneal administered with 100 mg/kg of PHT every 24 h for four days. At the fourth day of treatment, the same sampling schedule as mentioned for group A was carried out. On day 5, doses of VPM and PHT and samples withdrawal were similar to group A. Both groups received 0.5 mL of saline solution subcutaneously every hour during the sampling in order to prevent hypovolemia.

These administration regimes lead both groups of animals to the same handling stress, but took into consideration different duration of PHT inductive actions on both enzymes and efflux transporters. Group A had less than 2 days of inductive effects, while group B had more than 3 days of induction, enough for observing overexpression of proteins (Alvariza et al., 2013; Maldonado et al., 2011).

Each animal belonging to both VPM treated groups was acting as its own control (the day before VPM administration), in order to assess the impact that the interaction could have had on the pharmacokinetics of PHT. This could be advisable since PHT half-life was reported (Lolin et al., 1994) to be comprised between 2.6 h (after the first day of treatment) and 7.7 h (after the fifth day of treatment) when daily dose of 100 mg/kg were intraperitoneal administered to Sprague-Dawley rats. Then, no accumulation because of remaining drug in the body could be expected. Practically, the concentration-time curves to be obtained for PHT might resemble single doses only affected by the actual status of enzymes and transporters expressions.

In order to evaluate metabolic changes, the main metabolite of PHT was also monitored [5-(p-hydroxyphenyl)-5-hydroxyphenyl-hydantoin: p-HPPH]

### Phenytoin and 5-(p-hydroxyphenyl)-5-hydroxyphenyl-hydantoin analysis

PHT and p-HPPH plasma concentrations were determined by a high performance liquid chromatography method, based on the procedure developed by Savio et al. (1991) with minor modifications. Ten microliters of internal standard solution (Nitrazepam [NTZ] 16 μg/mL in methanol) were added to 50 μL of plasma. The extraction was performed with 250 μL of ethyl acetate and vortexed for 1 min. After centrifugation, the supernatant was separated and dried under nitrogen stream at 37—40 °C. Dry residue was dissolved with 50 μL of mobile phase and 20 μL injected into a Dionex Ultimate 3000 Series chromatograph. A Phenomenex® Luna C18 (5 μm, 100 Å, 150 mm × 4.6 mm) column was used as stationary reversed 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: 2.8, 4.5, and 5.8 min for p-HPPH, PHT, and NTZ, respectively.

The HPLC method was linear between 0.5 (the lower limit of quantification: LLOQ) and 25.0 mg/L for PHT and between 0.12 (LLOQ) and 2.8 mg/L for p-HPPH. Inter and intra-day coefficients of variation (CVs) for PHT and p-HPPH were below 15% and the accuracy of the method was between 85 and 115%.

### Pharmacokinetic and statistical analysis of data

Areas under the plasma concentration-time curves (AUC) were calculated for PHT and p-HPPH from zero (time of PHT

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