PHARMACOKINETIC INTERACTION STUDY BETWEEN CLOPIDOGREL AND PHENYTOIN IN HEALTHY MALE RABBITS

Issam Abushammala*1, Alaa Abusoud2 and Kamal Fakher Abu Shammaleh3

1,2Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Al-Azhar University-Gaza, Gaza, P.O Box: 1277, Palestine.
3Mohammed Eldora Pediatric Hospital, Palestinian Ministry of Health, Gaza, Palestine.

ABSTRACT

Introduction: This study was conducted to investigate the effect of clopidogrel – CYP2 C19 inhibitor on the pharmacokinetics (PK) of phenytoin in healthy male rabbits. The main aim of this study is to determine the PK of phenytoin after its daily oral administration when concomitantly administered with cytochrome CYP2 C19 inhibitor clopidogrel. Methodology: An in-vivo parallel designed drug-drug interaction (DDI) study was conducted in healthy male rabbits. Twelve rabbits were divided into two groups. The first group (The control group) received phenytoin of 30 mg/Kg/day for 14 days. On the day fourteen, blood samples were collected according to the time schedule 0.0, 1.30, 3.0, 4.30, 6.0, 7.30, 9.0, 12.0, 24.0, 36.0 and 48.0 hours (h) after the last dose. Chemiluminescent enzyme immunoassay (CLEIA) was used to measure phenytoin concentration in serum. The second group (The tested group) received phenytoin of 30 mg/Kg/day for seven constitutive days and at day 8 clopidogrel of 1.1 mg/Kg/day was added to the phenytoin until the day fourteen, then blood samples were collected as in the first group at the same time schedule for 48 h. Non-compartmental analysis by using winnonlin was used to determine the different PK parameters such as Cmax, Tmax, AUC0-48, t1/2 and ke. Results: Significant increase were reported in the second group related to first group in Cmax, AUC 0-48 and t1/2. In the first group the parameters were: Cmax=11.15μg/ ml and AUC 0-48=174.09 μg.h/ml. In the second group the last parameters increased significantly to: Cmax=11.66 μg/ ml, AUC 0-48=198.1 μg.h/ml and significant decrease was also reported in ke. In the first group ke=0.029 h−1 and decreased to ke=0.0185h−1 in the second group. The values of t1/2 were increased from t1/2 = 25.63 h in the first group to
36.51 h in the second group. There was insignificant change in Tmax. **Conclusion:** Clopidogrel alters the PK parameters of phenytoin to a significant levels.

1. INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent seizures as a result of neuronal hyperexcitability.[1] The effect of phenytoin in modulating the voltage gated sodium channels activity affects both the excitatory and inhibitory neurons.[2] So, phenytoin suppresses the efficacy of neurotransmission by reducing the voltage gated sodium channel activity that prevents the seizure episodes.[3]

Phenytoin sodium is related to the barbiturates in chemical structure, but has a five-membered ring. Phenytoin has a narrow therapeutic range 10-20 µg/ml, weakly acidic nature pKa 8.31 and poor aqueous solubility 100µg/ml.[4]

Phenytoin crosses blood-brain barriers. Therapeutic dose of phenytoin range is 400-600mg/day. Phenytoin has a long half-life (t1/2) range 12-36 hours (h). Plasma protein binding percentage of phenytoin is 90 %, this high percentage has clinical importance because the usual determination of blood concentrations indicate total drug in serum.[5]

Phenytoin is an inducer of CYP 3A4 activity, and a substrate and inducer of CYP 2C9 and CYP 2C19. Phenytoin is hydroxilated and converted to para isomer of 5-hydroxy-phenyl-5-phenylhydantoin (HPPH) in liver and then excreted as glucoronide conjugate in urine. The main metabolic reaction is catalyzed primarily by CYP 2C9 in liver, which presents 70-90 % from the total clearance and secondary metabolize by CYP 2C19.[6]

CYP enzyme is a family of hemoprotein includes group of enzymes present in liver and or the tissues and plays an important role in drug metabolism in addition to metabolizing endogenous molecules like fatty acids and steroid hormones. When two drugs act as substrates, inducers or inhibitors of CYP and are administered together, drug interaction may occur. Meanwhile, CYP 2C9 enzyme accounts around 20% of hepatic total CYP content and metabolizes around 15% of clinical drugs.[7,8]

Many enzymes expressed in liver and intestine are responsible on the oxidative drug metabolism such as CYP. Many approaches are provided to predict the magnitude of in vivo DDI based on the inhibition mechanism.[9]
Clopidogrel is an antiplatelet drug that used for the management and prophylaxis of cardiovascular thromboembolic events. Clopidogrel bisulfate, an inactive thienopyridine prodrug is 85% hydrolyzed in vivo by esterases to an inactive carboxylic acid derivative. The remaining drug undergoes oxidative biotransformation to its active thiol metabolite by a 2-step, CYP-dependent process in which CYP 3A4/5 and CYP 2C19 has the greatest roles, with lesser involvement from CYP 2B6, CYP 1A2, and CYP 2C9.\[^{10}\] The active metabolite then irreversibly inhibits the platelet P2Y12 ADP receptor by forming an inactivating disulfide bond with cysteine on the P2Y12 receptor. Clopidogrel is rapidly absorbed after oral administration of repeated doses of 75-milligram (mg) clopidogrel base, its oral absorption 70%, bioavailability 50%, protein binding 94%, metabolized by CYP 2C19 and standard daily dose is 75 mg. The bioavailability of clopidogrel may be diminished because of secretion of the active drug by an efflux pump P-glycoprotein (P-gp) encoded by the multidrug resistance gene. The PK of the main circulating metabolite are linear and it has significant inhibition effect on platelet aggregation.\[^{10,11}\]

Epilepsy is a disease that needs drug medication to prevent seizure episodes on long term treatment. So DDI between phenytoin and concomitantly used drugs is possible, because the patient needs other medication to deal with other conditions that may affect him. At the same time, DDI can become a serious problem, when two administered drugs are substrates to the same CYP enzymes, this may lead to PK interaction at the level of drug disposition. These types of interactions lead to increased plasma level of one or two drugs making the use of same drugs not safe. Metabolism of drugs is a very important stage in the fate of the drug inside the body, because it prevents the accumulation and toxification with drugs. Drugs of narrow therapeutic window like phenytoin is affected with the other drugs when used concomitantly, especially because it is a chronic used drug.\[^{12}\] The uses of other drugs that inhibit the same enzymes which metabolize phenytoin like clopidogrel which may lead to elevation in phenytoin plasma levels, and consequently toxicity symptoms may appear. In this in vivo study of the effect of clopidogrel CYP 2C19 inhibitor on phenytoin that is metabolized by the same enzyme will be conducted. The main aim of this study is to determine the PK of phenytoin after its daily oral administration when concomitantly administered with CYP 2C19 inhibitor clopidogrel.
2. MATERIALS AND METHODS

2.1 Study Design
An in-vivo DDI study was conducted in healthy male rabbits between clopidogrel and phenytoin. A parallel design studies for the two groups of rabbits were conducted. Twelve rabbits were divided into two groups six rabbits for each. The first group (The control group) received phenytoin 30 mg/Kg alone and the second group (The tested group) received phenytoin 30 mg/Kg with clopidogrel 1.1 mg/Kg. Comparative study was conducted between the two groups, where drugs were given at the same conditions. For this purpose, a PK method was selected to determine different PK parameters as $C_{\text{max}}$, $T_{\text{max}}$, AUC $0\rightarrow 48$, $t_{1/2}$, $k_e$.

2.2 Animals
Twelve randomly selected healthy male adult rabbits with weights ranged between 3.0 to 3.4 kg were included in the study. The rabbits were kept under standard animal housing conditions and were allowed to get water add libitum and free access of standard food. The rabbits were maintained in fasted conditions 12 hours with free access to water (add libitum) before administration of the last dose. Then blood samples were collected.[13] Topical lignocaine gel was applied on to minimize pain to the animals. Veterinary doctor checked the animals before, during and at the end of the study. The normal life conditions for the animals were kept based on the International Animal Ethics Committee.

2.3 Experimental Process
In the first group, the phenytoin was administered daily as free solution prepared from epanutine 100 mg capsules by using special oral gavage and the dose given was 30 mg/kg/daily for each rabbit. At day 14$^\text{th}$ of the experiment, rabbits (n=6) were fasted 12 h before experiment with free access to water then give them the last dose at the same daily time, after that the rabbits were putted in rabbit supporter boxes. Installation of IV-cannula to the ear marginal vein for each rabbit by a specialist nurse after shaving the ear hair by using suitable shaving cream and topical application of local anesthesia 5% lidocain gel. Collection of 1 ml of blood samples at the day fourteen in labeled vacutainer tubes according to the time schedule 0.0, 1.30, 3.0, 4.30, 6.0, 7.30, 9.0, 12.0, 24.0, 36.0 and 48.0 h after giving the last dose. Centrifugation of blood samples at 3500 rpm for 10 minutes to separate serum. Transfer serum in a clean glass tubes and kept in deep freezer at-20°C until be analyzed and assayed for phenytoin by Immulite1000 analyzer, while the second group started with the administration of the similar dose of phenytoin as in the first group for seven consecutive
days and at the day 8 till day fourteen, the phenytoin (30mg/kg/day) was given orally concomitantly with clopidogrel (1.1mg/kg/day) and the blood samples were collected as in the first group according to the time schedule 0.0, 1.30, 3.0, 4.30, 6.0, 7.30, 9.0, 12.0, 24.0, 36.0 and 48.0 h after giving the last dose. Centrifugation of blood samples at 3500 rpm for 10 minutes to separate serum. Transfer serum in clean glass tubes and keep it in deep freezer at -20°C until analyzing and assaying for phenytoin by Immulite1000 analyzer.

2.4 Analytical Procedures
Analysis of rabbit serum samples were made to determine the concentration of phenytoin at laboratory of Medical Relief Society-Gaza using phenytoin detection kit by using Immulite 1000 analyzer. The Kit was used for rapid detection of phenytoin concentrations in serum. This type of Kit is used normally in hospitals for rapid and routinely assay of phenytoin in blood as an aid in therapeutic drug monitoring.[14,15] An approval to conduct the study was obtained from Pharmacy College Committee.

2.5 Data Analysis
The PK parameters were calculated. The parameters of $C_{\text{max}}$ and $T_{\text{max}}$ were calculated directly from the plasma level data. Constant rate for plasma drug elimination i.e. $K_e$ was calculated by regression analysis of the mono exponential declining line of the plasma drug concentration versus time curve, while elimination $t_{1/2}$ was obtained from the formula, ($t_{1/2} = 0.693/K_e$). $\text{AUC}_{0-48}$ was calculated by trapezoidal rule. In this study, Win Nonlin 9 program was used to estimate the different PK parameters of the two groups. The data obtained experimentally was treated and analyzed by using statistical package of social science (SPSS) program version 16. Several statistical methods were conducted such as descriptive analysis (mean, standard deviation, coefficient of variation) and paired samples t-test. P-value < 0.05 was considered as statistically significant.

3. RESULTS
Table 1 shows paired test t-test for quality between mean PK parameters ($C_{\text{max}}$, $t_{\text{max}}$, $K_e$, $t_{1/2}$, $\text{AUC}_{0-48}$ and $\text{AUC}_{0-\infty}$) of phenytoin after administration of phenytoin solution 30 mg/kg alone (Reference group) and when co-administered with clopidogrel solution 1.1 mg/kg (Tested group) in rabbits. The table shows that there are statistically significant differences in PK parameters of phenytoin when phenytoin was administered alone and when co-administered with clopidogrel solution. 1mg/kg for the following parameters $C_{\text{max}}$, $K_e$, $t_{1/2}$, $\text{AUC0}_{-48}$ and $\text{AUC0}_{-\infty}$.
Table 1: Paired-samples t test for the equality between the means of the PK parameters of phenytoin alone (Reference group) and in presence of clopidogrel the (Tested group) for the six rabbits.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Difference</th>
<th>T</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$</td>
<td>Reference group</td>
<td>6</td>
<td>11.150</td>
<td>0.5434</td>
<td>5</td>
<td>-2.789</td>
<td>0.038*</td>
</tr>
<tr>
<td></td>
<td>Tested group</td>
<td>6</td>
<td>11.660</td>
<td>0.8133</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Reference group</td>
<td>6</td>
<td>25.632</td>
<td>9.7008</td>
<td>5</td>
<td>-4.570</td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td>Tested group</td>
<td>6</td>
<td>36.510</td>
<td>5.8157</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_e$</td>
<td>Reference group</td>
<td>6</td>
<td>0.0290</td>
<td>0.00704</td>
<td>5</td>
<td>4.704</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Tested group</td>
<td>6</td>
<td>0.0185</td>
<td>0.00176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AUC_{0-48}$</td>
<td>Reference group</td>
<td>6</td>
<td>174.09</td>
<td>20.33</td>
<td>5</td>
<td>-2.885</td>
<td>0.034*</td>
</tr>
<tr>
<td></td>
<td>Tested group</td>
<td>6</td>
<td>198.44</td>
<td>35.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AUC_{0-\infty}$</td>
<td>Reference group</td>
<td>6</td>
<td>190.53</td>
<td>35.479</td>
<td>5</td>
<td>-4.149</td>
<td>0.009*</td>
</tr>
<tr>
<td></td>
<td>Tested group</td>
<td>6</td>
<td>245.35</td>
<td>61.479</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant statistical difference (p ≤ 0.05)

A plot of an overlap mean serum concentration-time profile of phenytoin after administration of phenytoin solution 30 mg/kg alone and when co-administered with clopidogrel solution 1.1 mg/kg is illustrated in figure 1. The curves obtained in both cases obviously comparable.

Figure 1: A plot of the serum concentrations of phenytoin versus time after intake of phenytoin 30mg/kg (Reference group) co-administered with clopidogrel solution 1.1 mg/kg (Tested group) for the six rabbits.

4. DISCUSSION

Phenytoin is a widely used drug for treatment of epilepsy due to its low cost and good efficacy in preventing epileptic seizures. Phenytoin has a narrow therapeutic index,
metabolized mainly by CYP 2C9 and 2C19, has a high protein binding affinity of about 90% that when be used of other drugs concomitantly carry the risk of increase or decrease in the phenytoin concentrations in plasma which can lead to toxicity or loss of therapeutic effect of phenytoin in seizure control.\cite{1,6} Many enzymes expressed in liver and intestine are responsible on the oxidative drug metabolism such as CYP. Many approaches are provided to predict the magnitude of in vivo DDI based on the inhibition mechanism.\cite{9} 

Clopidogrel is an antiplatelet agent, it has a high protein binding affinity 94%, it is metabolized by and has the ability to inhibit CYP 2C19. DDI may occur when clopidogrel used with phenytoin at the same time due to the activity of clopidogrel as CYP 2C19 inhibitor.\cite{10} The utility of rabbit as a model to study drug–drug interactions is well documented.\cite{16,17} All twelve rabbits had completed the study without any deviations and there were no death or replacement during the study. The present study showed good tolerability of both formulations.

From the statistical treatment, a significant increase in C\text{max} was observed, the mean C\text{max} for the six rabbits was elevated from 11.15 µg/ml in the control group to 11.66 µg/ml in the tested group and also the t\text{1/2} was increased from 25.63 hours to 36.51 hours with P-value=0.038 and 0.006 consecutively for the control and tested group. The level of significance (P-value) was less than 0.05 for both treatments. The results proved that the delay of metabolism was as a result of CYP 2C19 inhibition in presence of clopidogrel.

**5. CONCLUSION**

Clopidogrel alters the PK of phenytoin to significant levels; because of the inhibition of CYP 2C19 which is the metabolizing enzyme of phenytoin. Adjustment of phenytoin plasma levels is very important when the patient takes clopidogrel at the same time to avoid toxic effects of phenytoin as a result of increase of phenytoin plasma levels, and avoiding the unwanted side effects.

**ACKNOWLEDGMENT**

The authors would like to thank Mr. Mohammed Abuaffash, the director of medical relief - Gaza for his invaluable assistance and analysis.
REFERENCES


15. Lequin R. "Enzyme Immunoassay (EIA)/Enzyme-linked immunosorbent assay (ELISA)", Clinical Chemistry, 2005; 2415-2418.
