EFFECT OF ESOMEPROAZOLE ON PHARMACOKINETICS OF PHENYTOIN IN RABBITS

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Abstract: Esomeprazole is commonly prescribed proton pump inhibitor for gastritis and peptic ulcer disease. Most of the time in clinical practice, phenytoin and esomeprazole are prescribed for patients of generalized seizures with concomitant peptic ulcer. Hence there are chances of drug-drug interaction because of modulations of isoenzymes CYP2C9 and CYP2C19, are involved in metabolism of phenytoin and esomeprazole. But it is important to maintain the therapeutic level of phenytoin in plasma for effective seizures control. So, the aim of the study was to determine the effect of esomeprazole on the pharmacokinetics of phenytoin in rabbits. In a parallel design study, phenytoin, 30 mg/kg/day per oral was given daily for 14 days. On day 15, blood samples were taken at various time intervals between 0-24 hours. In esomeprazole-phenytoin group, phenytoin was administered for seven days as mentioned earlier and from day 8th onward, esomeprazole 2.8 mg/kg along with phenytoin 30 mg/kg/day was administered till 14th days and blood samples were drawn as above on 15th day. Plasma phenytoin levels were assayed by HPLC and pharmacokinetic parameters were calculated. In esomeprazole-phenytoin group, there was a significant increase of t½el than phenytoin alone group and significant increase in AUC0-24 was also observed in the esomeprazole and phenytoin treated group. These results suggest that esomeprazole alters the pharmacokinetics of phenytoin. Confirmation of these results in further clinical studies will warrant changes in phenytoin dose or frequency when esomeprazole is co-administered.

Key words: phenytoin rabbits esomeprazole pharmacokinetics

INTRODUCTION

Epilepsy is frequently encountered during medical practice in a variety of clinical settings1,2. Presently, a large number of newer antiepileptic drugs are available in the market but still phenytoin is most widely prescribed antiepileptic. Because epilepsy requires long-term treatment, hence monitoring of the plasma concentration of

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these drugs is very crucial. Phenytoin is metabolized in the hepatic endoplasmic reticulum by isozymes CYP2C9 and CYP2C19. Phenytoin possesses a narrow therapeutic range beyond which toxic manifestations usually occur. Since the metabolism of phenytoin is saturable, other drugs that are metabolized by these enzymes can inhibit phenytoin metabolism leading to its raised serum concentration. Similarly, degradation rate of other drugs that are substrates for these enzymes can be inhibited by phenytoin. Moreover, the ability of phenytoin to induce diverse cytochrome P450 enzymes can lead to increased degradation of medications metabolized by these enzymes. Proton pump inhibitors (PPIs) are one of the most commonly prescribed medications in the primary care setting and are considered a major advance in the treatment of acid-peptic diseases. PPIs enabled improved treatment of various acid-peptic disorders, including gastroesophageal reflux disorder, peptic ulcer disease and nonsteroidal anti-inflammatory drug-induced gastropathy. These drugs have advantage of minimal side effects with negligible drug interactions, and are generally considered safe for long-term treatment. The proton pump inhibitors namely omeprazole, lansoprazole, rabeprazole, and the recently approved esomeprazole appear to have similar efficacy. Esomeprazole, the (S)-isomer of omeprazole, is the first PPI developed as a single isomer for the treatment of acid peptic diseases. Because of their extensive use, the documentation of the probability for drug interactions with esomeprazole is of great importance. Esomeprazole is extensively metabolized by the cytochrome P450 (CYP) enzyme system in the liver mainly by CYP3A4 and CYP2C19 isoenzymes. Depending on ethnic variations, metabolism of a drug by a particular cytochrome may predominate. Thus, the CYP2C19 isoenzymes is involved in the metabolism of both the drugs (even if less involvement in phenytoin) and hence, the potential of drug interactions between these two drugs may exists. Moreover, the high incidence of both gastrointestinal disorders and epilepsy, imply more chance to existence of one in the presence of the other. An episode of gastrointestinal problem in epileptic patients on phenytoin medication will necessitate the co-administration of esomeprazole, also. So, the purpose of the present study is to evaluate the effect of esomeprazole on the plasma concentration of phenytoin in rabbits.

MATERIALS AND METHODS

Experimental animals

Twelve randomly selected, healthy male New Zealand white rabbits, weighing between 1.5-2.5 kg were taken. The rabbits were kept under standard animal house conditions of 12:12 hour’s light/dark cycle at a temperature of 25±2°C and humidity of 60±2%. The animals were given free access to standard food and water ad libitum. The blood samples were withdrawn after application of topical lignocaine 4% to minimize pain to the animal. Injections were given as painlessly as possible. The study protocol was approved by the Institute Animal Ethics Committee (IAEC) of PGIMER, Chandigarh, India.

Drugs

Phenytoin (procured from Macleod’s
Pharmaceutical, Mumbai, India) and esomeprazole (procured from Lupin Pharmaceutical, Mumbai, India) were used in the study. These were in bulk powder form and were dissolved in appropriate solvents prior to administration.

**Procedure**

**Control group (Phenytoin)**: Six rabbits were administered phenytoin in a dose of 30 mg/kg/day, orally at 0800 hours for fourteen consecutive days using an oro-gastric tube. On day 14, blood samples (1 ml) were collected before administration of next dose of phenytoin at 0 hr and then at 0, 1, 2, 3, 4, 5, 6, 7, 9, 12 and 24 hours after drug administration.

**Phenytoin and esomeprazole group**: Six rabbits were administered phenytoin in a dose of 30 mg/kg/day orally at 0800 hours for seven consecutive days using an oro-gastric tube. After 7 days, esomeprazole, 2.8 mg/kg, was given orally with phenytoin (30 mg/kg/day) for next seven consecutive days. On day 15, blood samples were drawn at similar time intervals as mentioned above.

All blood samples were drawn from the marginal ear vein after topical application of anaesthesia with 4% lignocaine solution. Samples were collected in labelled, heparinised test tubes and centrifuged at 3000 rpm for 10 minutes. Plasma was separated by centrifugation and stored at −20°C until its assay for phenytoin by high performance liquid chromatography (HPLC) method.

**HPLC method for estimation of phenytoin**

Extraction procedure: To 0.2 ml plasma sample/standard sample, 0.2 ml of 1.0 M sodium acetate buffer (pH 5.5) and 3.0 ml of chloroform were added. The tubes were shaken for 1 min and then centrifuged at 3000 rpm for 10 minutes. Following this, 2.8 ml of chloroform layer was transferred in another test tube and the chloroform was evaporated on a water bath. The residue was reconstituted in 0.2 ml of mobile phase to be used for HPLC assay. 100 µl of this reconstituted solution was injected to HPLC system for assay.

**HPLC conditions**: The mobile phase containing acetonitrile: methanol: 4 mM potassium phosphate buffer (pH 6.0) in a ratio of 20:40:40(V/V/V) was delivered at a flow rate of 1.0 ml/min at ambient temperature. Absorbance was measured using a UV detector at 215 nm at a sensitivity of 0.02 AUFS. The sensitivity of the assay was 0.1 µg/ml with recovery 98% or more. The standards used for phenytoin ranged from 0.5 µg/ml to 32 µg/ml (8, 9, 10).

**Calculation of pharmacokinetic parameters**

Peak plasma concentration ($C_{max}$) and time to reach the peak plasma concentration ($T_{max}$) were read from the actual plasma level data. Rate constant for plasma drug elimination ($K_{el}$) was calculated by regression analysis of the monoexponential declining line of the natural log plasma drug concentration versus time curve, while elimination half life ($t_{1/2el}$) was obtained from the formula, ($t_{1/2el} = 0.693/K_{el}$). Absorption rate constant, $K_a$ was calculated by residual method. The absorption half life ($t_{1/2a}$) was calculated from the formula $t_{1/2a} = 0.693/K_a$. Area under the plasma drug concentration versus time curve (AUC_{0-24}) was calculated...
by trapezoidal rule. Statistical analysis was done using the unpaired student’s t-test to find the level of significance. P value ≤ 0.05 was considered significant.

RESULTS

Mean plasma levels of phenytoin were determined at different time intervals in both alone and in combination with esomeprazole groups. Significant changes in plasma levels of phenytoin were observed, when esomeprazole was given with phenytoin as compared to phenytoin alone control group (Fig. 1). Maximum plasma concentration (C_max) of phenytoin was significantly increased following concomitant administration with esomeprazole. After the combination, the time to reach maximum plasma concentration (T_max) also increased significantly. Esomeprazole significantly prolonged the absorption of phenytoin, and the absorption constant (K_a) was found to be (0.304 vs 0.565) whereas t½a was significantly increased (1.2 hr⁻¹ vs 2.3 hr⁻¹). The elimination half life of phenytoin, (t½el) (37.9 vs 55.7 hr) and the area under the curve (AUC_0-24) (10.88 vs 326.95 μg/ml.h) increased significantly when it was combined with the esomeprazole as compared to phenytoin alone. There was 35 fold increase in the AUC_0-24 of phenytoin in the combination group as compared to phenytoin alone group (Table I).

![Fig. 1](Phenytoin plasma levels (mean±SD) at different time intervals of phenytoin alone and after oral esomeprazole administration. Values are mean±SD, (n=6), *P<0.05 (unpaired ‘t’-test).)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Phenytoin (Control)</th>
<th>Phenytoin+ Eosomeprazole group</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (µg/ml)</td>
<td>12.8±1.36</td>
<td>44.0±3.06*</td>
</tr>
<tr>
<td>T_max (hrs)</td>
<td>4.0±0.54</td>
<td>6.0±0.87*</td>
</tr>
<tr>
<td>K_a (hr⁻¹)</td>
<td>0.565±0.078</td>
<td>0.304±0.037*</td>
</tr>
<tr>
<td>t½a (hr)</td>
<td>1.2±0.41</td>
<td>2.3±0.82</td>
</tr>
<tr>
<td>Kel (hr⁻¹)</td>
<td>0.018±0.009</td>
<td>0.012±0.003</td>
</tr>
<tr>
<td>t½el (hr)</td>
<td>37.9±3.51</td>
<td>55.7±7.16*</td>
</tr>
<tr>
<td>AUC_0-24 (µg/ml.h)</td>
<td>10.88±2.75</td>
<td>326.95±31.62*</td>
</tr>
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*P<0.05; Unpaired t’test use for the statistical analysis.

DISCUSSION

Phenytoin is preferred drug among older antiepileptics due to low cost and efficacy against a range of syndromes. Since, it has a narrow therapeutic index, the monitoring of phenytoin level is a common practice in clinical set up for the intervention of patients. Due to its long list of drug-drug interactions, phenytoin has chances to interact with the other concomitant drugs. The patients suffering from epilepsy can have concurrent occurrence of some other diseases (3, 11). Phenytoin is given for a long duration; hence its pharmacokinetics may be altered by drugs indicated in other disease conditions. This may lead to an increase or decrease in its plasma levels and subsequent deleterious effects either due to toxicity or loss of effective seizure control by this antiepileptic (11). Present
study was carried out to evaluate possible pharmacokinetic interaction of esomeprazole on concomitant use with phenytoin. Rabbits were used in the study, since it is considered as an ideal animal for pharmacokinetic studies because of good sensitivity as well as ease of multiple sampling which is required for such studies. Phenytoin and esomeprazole dose calculations were based on extrapolation of human recommended doses to rabbits using conversion factor (12). The dose used for phenytoin has been successfully used in similar experiments in our laboratory (8-10). The dose regimen for phenytoin was based on pilot studies, which showed no significant difference in plasma concentrations of phenytoin after 7 and 14 days of administration of phenytoin in adult healthy male rabbits (8-10, 12). Dose calculation of esomeprazole was also based on recommended human treatment regimes so as to closely mimic human situations of use. Since, both drugs (esomeprazole and phenytoin) were administered orally, there may chances of interaction in transport processes of both the drugs. The consequent increase in the fraction of unbound phenytoin in the plasma results in only a transient increase in the effect of phenytoin because it results in a proportionately increased rate of metabolism of phenytoin (14, 15).

Present study showed with esomeprazole increases the elimination half life & AUC of phenytoin significantly, as the data indicate prolonged extent of absorption of phenytoin with esomeprazole, with increases mean plasma levels of phenytoin compared to phenytoin alone group, since the rate of elimination of phenytoin is prolonged by 47.2% compared to control group. So it may be clinically relevant in actual practice where above the therapeutic level can lead to adverse effects. In such a situation, the dose of phenytoin will have to adjust to therapeutic levels to maintain adequate control of seizures in order to avoid unnecessary adverse drug reactions.

The metabolism of phenytoin is non linear within the therapeutic range because the enzyme system responsible gradually becomes saturated at relatively low plasma phenytoin concentration (within the therapeutic range), resulting in a progressive decrease in the rate of elimination of phenytoin as the plasma level is increased (13). It has been found that, there is significant increase in rate of elimination of phenytoin with esomeprazole (14, 16).

In conclusion, esomeprazole alters the pharmacokinetics of phenytoin to a significant level, since it is a competitive inhibitor of CYP2C9 and CYP2C19 (16, 17). The results of this study need to be confirmed in drug interaction studies in humans, to warrant a recommendation for altering dosage of phenytoin in a patients on chronic therapy for epilepsy who requires esomeprazole as a concomitant treatment for the management of peptic ulcer disease.

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