Study of the fluvastatin effect on phenytoin disposition in healthy male rabbits

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Abstract

Introduction*:* Drug-drug interaction is a serious problem facing patients with chronic diseases like epilepsy; it is also an important reason for drugs refusal and withdrawal from markets. That makes the study and evaluation of the probability of drug-drug interaction is an important research area. Phenytoin is one of antiepileptic drugs which are widely used for seizure relieve. Fluvastatin is a lipid lowering drug that acts by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that makes fluvastatin as a potent lipid lowering drug and prevents cardiovascular diseases. The goal of this study was to evaluate the effect of Fluvastatin on daily oral administered phenytoin pharmacokinetic in therapeutic dose and studying the changes in phenytoin serum concentrations when adding oral Fluvastatin to Rabbits.

Materials and Method: An in-vivo drug-drug interaction study was conducted in healthy male rabbits between fluvastatin and phenytoin. Parallel designed studies for the two groups of rabbits were conducted. Twelve rabbits were divided into two groups. The first group (control group) received phenytoin of 25 mg/Kg/day for 14 days. On the day 15, blood serum samples were collected over a period of 24.0 hours after the last dose according to the designed time schedule 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 12.0 and 24.0 hours. The second group (tested group) received phenytoin of 25 mg/Kg/day for seven consecutive days and at day 8 was added fluvastatin at 4 mg/Kg/day until day 14. On the day 15, blood serum samples were collected at the same time schedule as in the first group. Chemiluminescent enzyme immunoassay (CLEIA) was used to measure serum phenytoin levels. Non-compartmental analysis by using WinNonlin was used to determine the different pharmacokinetic parameters such as $C_{max,}$ $T_{max,}$ AUC $_{0\text{-}24}$, $t_{1/2}$ and k_e.

Results: Significant increases were reported in tested group related to reference group in C_{max} and AUC $_{0\text{-}24}$ of phenytoin. In control group the parameters were: C_{max}= 5.18 µg/ ml and AUC $_{0\text{-}24}$= 80.99 µg. h/ml. In the tested group these parameters increased significantly to: C_{max}= 6.96 µg/ ml and AUC $_{0\text{-}24}$= 121.93 µg. h/ml. In this group, significant decrease was also reported in k_e (0,019 h^{-1} vs 0,046 h^{-1} in control group). Moreover, the $t_{1/2}$ was doubled in tested group (41, 6 hours vs 20, 44 hours in control group). The decrease in T_{max} was insignificant, it decreased from 11.18 hours in the control group to 10 hours in the tested group.

Conclusion: Fluvastatin alters the pharmacokinetic parameters of phenytoin to significant levels.

Keywords: Phenytoin, Fluvastatin, Drug-drug interaction, Pharmacokinetic parameters, CYP2C9.

Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures as a result of neuronal hyper excitability.(1) It has prevalence rate of 4 to 10 per 1000 population.(2) Most epileptic patients receive multiple drugs for their all lives.(3) Phenytoin is a commonly prescribed antiepileptic drug used for management of both generalized and partial epilepsy, in the chronic and acute management of all seizure disorders, except absence seizures.(4) Phenytoin is an inducer of Cytochrome P450 (CYP450) subtype CYP3A4 activity, and it is a substrate and inducer of CYP2C9 and CYP2C19. Phenytoin is hydroxilated and converted to para- isomer of 5-hydroxy-phenyl-5-phenylhydantoin in liver and then excreted as glucoronide conjugate in urine.(5) The main metabolic reaction is catalyzed primarily by CYP2C9 in liver, which represents 70-90% from the total clearance and secondary metabolism by CYP2C19.(6)

The use of phenytoin makes the drug-drug interaction as an expected event when the patient uses other drugs concomitantly, especially when the used drugs affect the metabolizing enzymes of phenytoin.(5) Phenytoin is characterized by a narrow therapeutic range which makes the drug-drug interaction as an expected event. Phenytoin has a saturation pharmacokinetics on the metabolic level, which exhibits non-linear or dose dependent kinetics, that means when phenytoin dose is increased by 50% in patient from 300 mg to 450 mg, the average steady-state plasma concentration may increase by as much as 10-folds.(7)

Fluvastatin is a lipid lowering drug considered as HMG-CoA reductase enzyme inhibitor that makes fluvastatin as a potent lipid lowering drug and prevents cardiovascular diseases. Recent researches show different new uses of fluvastatin that makes it a promising drug in many medical fields. Fluvastatin is also a substrate and potent inhibitor of CYP2C9.(8,9) In addition of the lipid lowering effects of Fluvastatin, there are many recent studies focused on other different effects of fluvastatin in medicine. Fluvastatin is extensively used in medical research practices like, fluvastatin anti-flushing effects in menopausal women,(10) suppression of cancer growth and development,(11) reduction effect of low-density lipoprotein,(12) and improvement of microcirculation.(13)

Drug-drug interaction is a serious problem facing patients with chronic diseases like epilepsy; it is also an important reason for drugs refusal and withdrawal from markets.[14]

The serum phenytoin concentrations were determined by immunoassay technique. This assay gives accurate concentrations of the phenytoin in serum. Immunoassay techniques are chemical tests used to detect and quantify the low concentrations of drugs, hormones and proteins in body fluids. They are highly sensitive and accurate[15] and recently are widely used as in vitro diagnostic technique in medical researches.[16]

Materials and Method

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 according day/night cycle and under temperature of 25 $\pm$ 2ºC. The animals were allowed to get water *add libitum* and free access of standard food. At the end of the experiment, the food were taken away to let the animals in fasted conditions at last 12 hours with free access to water (*add libitum*) just before administration of the last dose. Then blood samples were collected. Topical lidocain gel was applied on the marginal vein in rabbit's ears to minimize pain of 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. There are many advantages for using rabbit as animal model because this animal is very docile and non-aggressive and hence easy to handle and observe, very economical and have short life cycles. The best size of rabbit is 3-3.5 Kg it can withstand experimental conditions and has better survival rate.[17] Rabbits showed good sensitivity in pharmacokinetic studies in addition to simple and suitable for multiple sampling that is required for pharmacokinetic studies. That made rabbits as ideal animals for such studies among small mammalian animals.[18,19]

Study design: An in-vivo drug-drug interaction study was conducted in healthy male rabbits between fluvastatin and phenytoin. A parallel design studies for the two groups of rabbits were conducted. Twelve rabbits were divided into two groups. The first group (control group) received phenytoin 25 mg/ Kg alone for 14 days. At day 15 blood samples were collected. The second group (tested group) received phenytoin 25 mg/ Kg alone for the first 7 days. From day 8, fluvastatin was added at 4 mg/ Kg, concomitantly with phenytoin until day 14. At day 15 blood samples were collected (Medhi et al., 2012). Comparative study was conducted between the two groups, where drugs were given at the same conditions and the following different pharmacokinetic parameters as C_{max}, T_{max}, AUC $_{0-24}$, $t_{½}$ and k_e were determined.

Blood sampling: Rabbits were fasted 12 hours before giving the last dose 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. IV-cannula in the ear after shaving the hair were installed and 5% lidocine gel as local anesthetic were applied. At 15 day, 1 mL of blood was collected in vacutainer tubes according to the designed time schedule 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 12.0 and 24.0 hours after giving the last dose. Samples at 0.0 times were taken just prior to administering the dose. The samples were centrifuged at 3500 rpm for 10 minutes to separate serum. The serum was transferred to a clean glass tubes and kept in deep freezer at -20ºC until be analyzed and assayed for phenytoin by Immulite1000 analyzer.

Analysis of serum samples: Rabbit serum samples were analyzed in Medical Relief Society-Gaza. The analytical method was enzyme multiplied immunoassay technique (EMIT) by using Immulite ® 1000 analyzer, Siemens.

Pharmacokinetic analysis: Pharmacokinetic parameters of phenytoin for both groups, including maximum plasma drug concentration (C_{max}), time to reach C_{max} (T_{max}), area under the plasma concentration time curve (AUC), were estimated by standard non-compartmental methods. Area under the plasma drug concentration versus time curve (AUC $_{0-24}$) was calculated by trapezoidal rule.

Pharmacokinetic analysis was performed by means of model independent method (Non-Compartmental Approach) Win Nonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC).

Statistical analysis: The data obtained experimentally was treated and analyzed by using the Statistical package of social science (SPSS) program version 16 by using Paired samples t-test and P-value < 0.05 considered as statistically significant.

Results

The mean and standard deviation of phenytoin pharmacokinetic parameters, administered alone or in combination with fluvastatin, as well as the statistical significance following their comparison are given in Table 1. The concentration time profile obviously indicated that the two periods are comparable.

In control group the parameters were: C_{max}= 5.18 µg/ ml and AUC $_{0-24}$= 80.99 µg. h/ml. In the tested group both parameters increased significantly to: C_{max}= 6.96 µg/ ml and AUC $_{0-24}$= 121.93 µg. h/ml. In tested group, significant decrease was also reported in k_e (0,019 h^{-1} vs 0,046 h^{-1} in control group). The values of $t_{1/2}$ were increased approximately to the double. It increased from $t_{1/2}$= 20.44 hours in control group to $t_{1/2}$=41.6 hours in tested group. The decrease in T_{max} was insignificant, it decreased from 11.18 hours in the control group to 10 hours in the tested group.

The obtained results demonstrated that the fluvastatin in a significant way, inhibit the CYP2C9 which is the major metabolizing enzyme of phenytoin.

Table 1: Paired-samples t-test for the equality between the means of the pharmacokinetic parameters of phenytoin alone (control group) and the (tested group) in the presence of fluvastatin

Parameter	Group	No. of rabbits	Mean	Unit	Standard deviation	Difference	T	P-value
C_{max}	Control Group	6	5.18	µg/ ml	1.3389	5	-2.896	0.034*
	Tested group	6	6.96	µg/ ml	1.2997			
t_{max}	Control Group	6	11.18	hours	2.0424	5	1.337	0.239
	Tested group	6	10.00	hours	1.5494			
AUC_{0-24}	Control Group	6	80.99	µg. h/ml	19.8693	5	-4.108	0.009*
	Tested group	6	121.93	µg. h/ml	19.3746			
Ke	Control Group	6	0.046	h^{-1}	0.01911	5	3.726	0.014*
	Tested group	6	0.019	h^{-1}	0.0097			

* Significant statistical difference ($p \leq 0.05$)

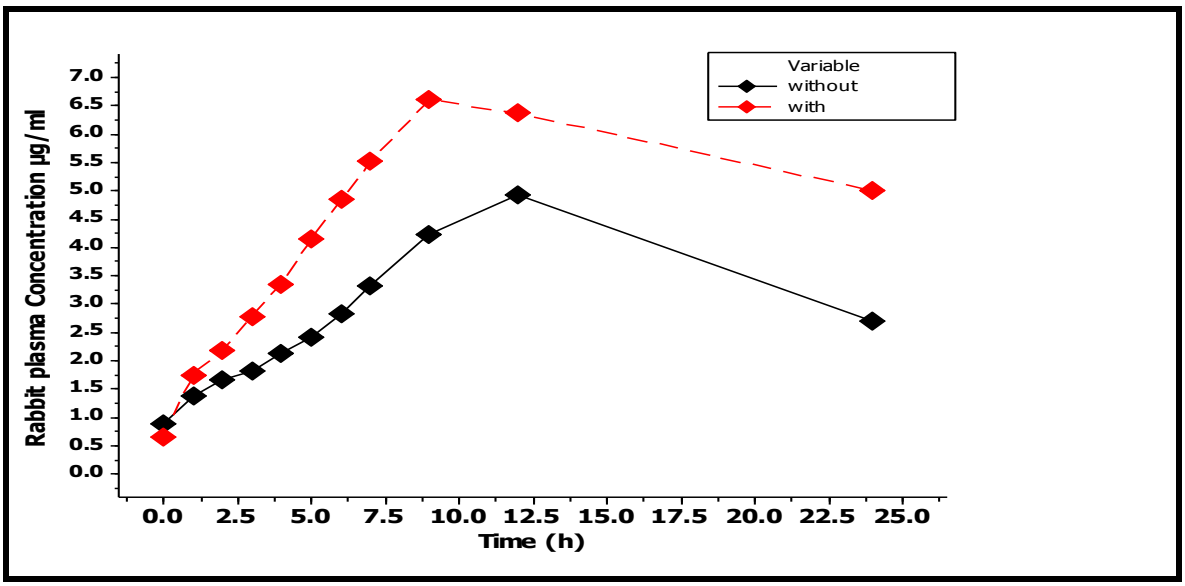

Fig. 1: A plot of the mean concentrations of serum phenytoin versus time after intake of phenytoin 25 mg/ Kg without fluvastatin (black curve) in control group, and when co-administered with fluvastatin 4 m/ Kg for the six rabbits (red curve) in tested group

Discussion

Phenytoin is a widely chronic used drug for treating epilepsy due to low cost and efficacy in preventing epileptic seizures. Phenytoin has narrow therapeutic index that makes drug-drug interaction is a serious problem when using other drugs concomitantly. This leads to toxicity or loss of effect in seizure control.[7]

Rabbits were ideal animals for studying the pharmacokinetic parameters and drug-drug interaction between phenytoin and fluvastatin. They were easy to handle for giving multiple oral doses of drugs and for the extraction of blood samples.[18,19]

CLEIA technique which were used to estimate the serum levels of phenytoin different times after administration was very convenient and time efficient.[15,16]

Both phenytoin and fluvastatin have a high degree of plasma protein binding. That allows evaluating the effect of drug-drug interaction between phenytoin and fluvastatin focusing on metabolism without interference of protein binding.

From the statistical treatment, a significant increase in C_{max} was observed, the mean C_{max} was elevated from 5.18 µg/ml in the control group to 6.96 µg/ml in the test group. The level of significance (P- value) was less than 0.05. In the t_{max} statistical treatment, it was obtained that P-value= 0.239 that means insignificant decrease in t_{max} were observed. The last results proved that the delay of metabolism was as a result of CYP2C9 inhibition in presence of Fluvastatin.[19]

Significant decrease in k_e was observed and that refer to the inhibition of CYP2C9 enzyme that responsible on the phenytoin metabolism inside the body. In the control group k_e was 0.046 h^{-1} and decreased to 0.019 h^{-1} in the tested group, that decrease was significant with P-value=0.014.

Significant increase in AUC_{0-24} was reported. The AUC_{0-24} changed from 80.99 µg.h/ml in the control group to 121.93 µg.h/ml in the tested group with p-value=0.009. This increase is related to the decrease in k_e and the accumulation of phenytoin inside the body. This results was similar to the esomeprazole and phenytoin drug-drug interaction applied by (20), also similar results reported in the drug-drug interaction between fluconazole and phenytoin applied by (21).

The increase in the $t_{1/2}$ of phenytoin from 20.44 hours in the control group to 41.61 hours in the tested group refer to the phenytoin metabolism inhibition which lead to phenytoin accumulation inside the body in presence of the CYP2C9 inhibitor fluvastatin. The zero order elimination kinetics of phenytoin was represented in the delayed metabolism and accumulation of phenytoin at 24 hours point. The last accumulation of phenytoin was the reason of the long $t_{1/2}$. The presence of fluvastatin was inhibited the enzymes that already saturated with phenytoin which leaded to further increase in the $t_{1/2}$ to approximately the double. Similar results was reported in the esomeprazole and phenytoin metabolic interaction that applied by (20). Other similar results were reported in amiodarone and phenytoin drug-dug interaction applied by (22).

Conclusion

Fluvastatin alters the pharmacokinetics of phenytoin in a significant way; through the inhibition of CYP2C9, which is the major metabolizing enzyme of phenytoin.

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