

Effect of aceclofenac on pharmacokinetic of phenytoin

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Abstract: Aceclofenac is presently most commonly prescribed analgesic for chronic pain and inflammatory conditions. In clinical practice, phenytoin and aceclofenac are used in a chronic condition of generalized seizure with concomitant chronic pain. Hence there are chances of drug-drug interaction because modulations of isoenzymes involved in metabolism of phenytoin and aceclofenac are CYP2C9/10 and CYP2C19. It is important to maintain the therapeutic level of phenytoin in plasma for effective control of seizure. So, the aim of the study was to determine the effect of aceclofenac on the pharmacokinetics of phenytoin in rabbits. In a parallel design study, phenytoin 30 mg/kg/day per oral was given daily for seven days. On day 7, blood samples were taken at various time intervals between 0-24 hours. In aceclofenac group, phenytoin was administered for seven days as above. On day 8, aceclofenac 14 mg/kg alongwith phenytoin 30 mg/kg/day was administered and blood samples drawn as above. Plasma phenytoin levels were assayed by HPLC and pharmacokinetic parameters were calculated. In aceclofenac group, there was decrease in $t_{1/2}$ el than phenytoin group significant changes were observed in the pharmacokinetic parameters in aceclofenac treated group. These results suggest that aceclofenac alter the pharmacokinetics of phenytoin. Confirmation of these results in human studies will warrant changes in phenytoin dose or frequency when aceclofenac is co-administered with it.

Keywords: Phenytoin, aceclofenac, pharmacokinetics.

INTRODUCTION

Epilepsy affects more than 50 million people worldwide, 5 million of whom have seizures more than once per month (Porter, 1988). Although a variety of factors influence the incidence and prevalence of seizures, approximately 5 to 10% of the population will have at least one seizure during their lifetime, with highest incidence occurring in early childhood and late adulthood. Because seizures are common, the clinical problem is frequently encountered during medical practice in a variety of clinical settings (Lowenstein, 2001, Sohi *et al.*, 1993).

Phenytoin is most widely prescribed antiepileptics. Epilepsy requires long-term treatment and monitoring the plasma concentration of these drugs is useful. Phenytoin is metabolized in the hepatic endoplasmic reticulum by CYP2C9/10 and CYP2C19. The effects of phenytoin occur over a narrow therapeutic range beyond which toxic manifestations occur. Because its metabolism is saturable, other drugs that are metabolized by these enzymes can inhibit phenytoin metabolism and raise its serum concentration. Conversely, degradation rate of other drugs that are substrates for these enzymes can be inhibited by phenytoin. Also, the ability of phenytoin to induce diverse cytochrome P450 enzymes can lead to increase degradation of medications metabolized by these enzymes.

A newer NSAIDs aceclofenac, a phenylacetic acid derivative with anti-inflammatory and analgesic properties is most commonly prescribed in clinical practice. After oral administration of a single 100 mg dose, mean maximum plasma aceclofenac concentration (C_{max}) of 6.8 to 8.9 mg/L were reduced in about 1.4 to 2 hours. C_{max} and area under the plasma concentration time curve (AUC) increase linearly after administration of single dose of aceclofenac 50, 100 and 150 mg and the pharmacokinetic properties of the drug were generally unchanged during multiple-dose administration in both young and elderly volunteers. Aceclofenac is mainly metabolized by CYP2C9 pathway (Rex *et al.*, 1996, Bort *et al.*, 1996). Thus it is apparent that antiepileptic phenytoin shares some metabolic pathway with aceclofenac. Depending on ethnic variation, metabolism of drug by a particular cytochrome may predominate. So the potential of drug interactions between these two groups drug exists. Moreover, high incidence of painful inflammatory disorders may need concomitant administration of antiepileptic with above mentioned drugs may result serious drug interaction. So far no study has been conducted to evaluate drug interaction of phenytoin with aceclofenac.

So the purpose of the present study is to evaluate any influential effect of aceclofenac on the plasma concentration of the antiepileptic phenytoin in rabbits.

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MATERIALS AND METHODS

Experimental animals

Twelve randomly selected healthy male white New Zealand rabbits weighing between 1.5 kg and 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 $25\pm 2^\circ\text{C}$, humidity of $60\pm 2\%$. The animals were allowed to take water *ad libitum* and free access to standard food. The blood samples were withdrawn after application of topical lignocaine 4% anaesthesia 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), Aceclofenac (procured from Emcure Pharmaceutical, Mumbai, India) were used in the study. These were in bulk powder form and were dissolved in appropriate solvents prior to administration.

Study design

A parallel design study was used. Total twelve male rabbits were selected and divided into two groups, phenytoin group and phenytoin with aceclofenac group. The weight of the rabbits was 1.5-2.5 kg and kept in the laboratory one week prior to the start of the experiment to acclimatize. The laboratory condition was kept standard at $23\pm 2^\circ\text{C}$ temperature and of 60% humidity. The rabbits were kept at standard food and tap water *ad libitum*.

Procedure

Control group (Phenytoin): 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. On day 7, 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 Aceclofenac 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, aceclofenac 14 mg/kg, was given orally with phenytoin (30 mg/kg/day) from 8th to 14th days and blood samples 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. Each sample was 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 assayed for phenytoin by high performance liquid chromatography

(HPLC) method. The plasma was isolated just after the blood withdrawal and plasma was stored at -80°C till the drug level assessment was done by HPLC.

HPLC method for estimation of phenytoin

Extraction procedure: To 0.2 ml plasma sample/standard sample 0.2 ml of 1.0 M of 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 min. 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 was acetonitrile: methanol: 4 mM potassium phosphate buffer (pH 6.0) in ratio of 20:40:40(V/V/V) delivered at a flow rate of 1.0ml/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 $\mu\text{g/ml}$ with recovery 98% or more. The standards used for phenytoin ranged from 0.5 $\mu\text{g/ml}$ to 32 $\mu\text{g/ml}$ (Aronson *et al.*, 1996).

DATA ANALYSIS

The pharmacokinetic parameters were calculated and following parameter, Peak plasma concentration (C_{max}) and time to reach the peak plasma concentration (T_{max}) were calculated from the actual plasma level data. Rate constant for plasma drug elimination i.e. K_{el} was calculated by regression analysis of the monoexponential declining line of the plasma drug concentration versus time curve, while elimination half life ($t_{1/2\text{el}}$) was obtained from the formula, ($t_{1/2\text{el}} = 0.693/K_{\text{el}}$). Absorption rate constant K_{a} was calculated by residual method. The absorption half life ($t_{1/2\text{a}}$) was calculated from the formula $t_{1/2\text{a}} = 0.693/K_{\text{a}}$. Area under the plasma drug concentration versus time curve (AUC_{0-t}) was calculated by trapezoidal rule. Extension of the AUC data to infinity ($\text{AUC}_{t-\infty}$) was done by dividing the last observed concentration in plasma by the elimination rate constant (K_{el}). The $\text{AUC}_{0-\infty}$ was calculated from the sum of AUC_{0-t} and $\text{AUC}_{t-\infty}$. Statistical analysis was done using the unpaired student's t-test to find the level of significance. P value ≤ 0.05 was considered statistically significant.

RESULTS

In the Control group, mean plasma levels were determined when phenytoin was given alone and the pharmacokinetic parameter calculated as described above. In group, aceclofenac mean plasma levels of phenytoin determined at different time intervals following aceclofenac oral administration. Significant changes in

plasma levels of phenytoin were observed when aceclofenac was given with phenytoin compared to phenytoin controls (table 1).

Table 1: Mean drug level reaching to the plasma

	Aceclofenac ($\mu\text{g/ml}$; Mean)	Phenytoin+ Aceclofenac ($\mu\text{g/ml}$; Mean)
0hr	0.030399	0.628
30min	0.419268	1.235
1hr	1.863089	1.235
3hr	2.601057	7.267
4hr	3.202685	8.037
5hr	3.26213	11.058
6hr	2.152033	8.81
9hr	0.627281	5.9
12hr	0.223244	3.73
24hr	0.132455	2.9

There were significant decrease in the maximum plasma concentration of phenytoin (C_{\max}), the time to reach

maximum plasma concentration (T_{\max}) was unchanged. The elimination half-life of phenytoin ($t_{1/2\text{el}}$) and the area under the curve (AUC_{0-24}) was decrease when aceclofenac was given alongwith phenytoin as compared to phenytoin alone. The decrease of AUC_{0-24} was 66% which is highly significant. There was a significant decrease of elimination rate of phenytoin in the aceclofenac treated group by approx 80% (table 2 and fig.).

DISCUSSION

Epilepsy is characterised by periodic and unpredictable occurrences of seizures (McNamara, 2001). It is a chronic disorder and requires long-term treatment. Phenytoin is an oldest drug used which is still preferred, since it has a narrow therapeutic index, so plasma concentration monitoring is often used to guide therapy. Many drug-drug interactions involving phenytoin have been reported. Some cases are those in which other drugs modify the pharmacokinetics of other drugs usually through its potent effect of inducing hepatic microsomal enzymes. There are chances that patients suffering from epilepsy can have

Table 2: Different pharmacokinetic parameters of phenytoin alone a following oral administration of aceclofenac

Pharmacokinetic parameters	Phenytoin group (Control)	Phenytoin + Aceclofenac group
C_{\max} ($\mu\text{g/ml}$)	11.05 ± 2.12	$3.26 \pm 1.42^*$
T_{\max} (hrs)	5.0 ± 0.88	5.00 ± 0.88
K_a (hr^{-1})	0.36 ± 0.06	0.47 ± 0.06
$t_{1/2\text{a}}$ (hr)	1.93 ± 0.22	1.48 ± 0.12
K_{el} (hr^{-1})	0.03 ± 0.03	$0.16 \pm 0.01^*$
$t_{1/2\text{el}}$ (hr)	21.83 ± 0.65	$4.34 \pm 0.61^*$
AUC_{0-24} ($\mu\text{g/ml.h}$)	60.22 ± 24.12	$20.81 \pm 22.02^*$

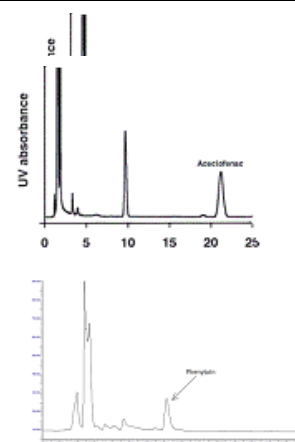
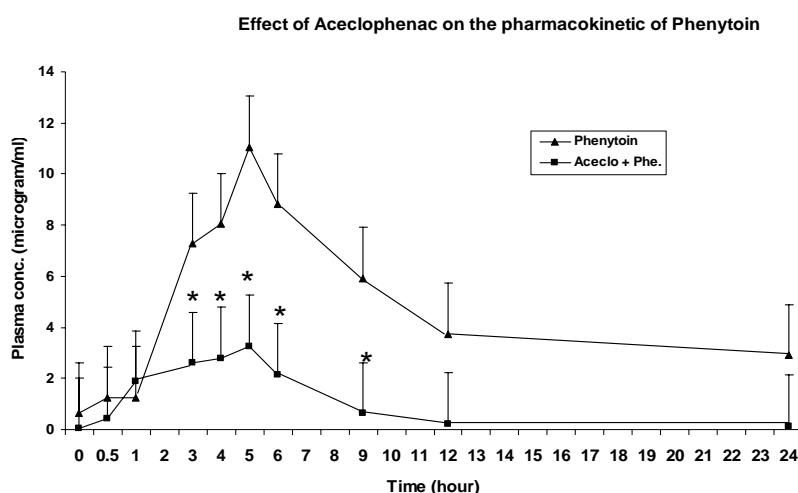


Fig. Phenytoin plasma levels (Mean \pm SEM) at different time intervals of phenytoin alone and after oral aceclofenac administration. Insight figure showed the standard chromatogram of aceclofenac and phenytoin. Values are Mean \pm SEM, (n=6), * $p < 0.05$; considered significant. Significance level was determined by using unpaired 't'-test.

concurrent occurrence of some other diseases. Since phenytoin is given for a prolong time, it is likely to be taken alongwith other drugs which 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 (Perucca, 2005).

Present study was carried out to evaluate whether any possible drug interaction that can occur if these drugs are concomitantly given with phenytoin. Rabbits are ideal animals for pharmacokinetic studies. They have been used extensively in this area and show good sensitivity as well as ease of multiple sampling which is required for such studies. Drug dose calculations are based on extrapolation of human recommended doses to rabbits using conversion factor (Ghosh, 2005). The dose used for phenytoin has been successfully used in similar experiments in our laboratory (Sidhu *et al.*, 2004, Sukhija *et al.*, 2006). 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 (Shekher *et al.*, 1997). Dose calculation of aceclofenac was also based on recommended human treatment regimes so as to closely mimic human situations of use.

Aceclofenac is a common COX-2 inhibitor used extensively in various conditions of arthritis like rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Rex *et al.*, 1996). Both aceclofenac and phenytoin are orally administered drugs. The gastrointestinal absorption of drugs is a complex process and the rate and extent of absorption depends on several factors like lipid solubility, formulations, splanchnic blood flow, metabolic capacity of the gastrointestinal tract and disease states (Bort *et al.*, 1996). Whenever two drugs are given orally there can be competition for transport processes involved in absorption. Aceclofenac is highly protein bound drug extensively with albumin. A study has been conducted to evaluate the mechanism of interaction of aceclofenac with human serum albumin (HSA) using fluorescence spectroscopy (Vargas *et al.*, 2007). It was found that aceclofenac is more than 99.7% bound to albumin. Phenytoin is also highly bound to albumin (90%) and it has been found that various NSAIDs like aspirin, ibuprofen, naproxen etc. has displaced the phenytoin from its binding sites on plasma albumin (Dasgupta *et al.*, 1996). 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 (Aronson *et al.*, 1996).

The metabolism of aceclofenac differs between different laboratory animals and the human being. Aceclofenac, additional side-chain (esterifies acetoxy) is compared with

structurally related diclofenac. 4' OH aceclofenac is the main metabolite in the plasma and urine after the oral administration of aceclofenac. Other minor metabolites are diclofenac and 4' OH diclofenac, 5'-OH diclofenac. Aceclofenac gets converted to diclofenac after long time. The minor metabolite diclofenac originate from the breakage of the ester bound by esterases where as 4'-OH diclofenac can be formed either as a consequence of diclofenac hydroxylate (CYP 2C9) or of hydrolysis of the present compound (4OH-Ace) (Bort *et al.*, 1996).

In our study we have found that aceclofenac has decreased the elimination half-life of phenytoin by 80% and the AUC of phenytoin was also decreased by 66%. It means aceclofenac has decreased the extent of absorption of phenytoin. Aceclofenac when given alongwith phenytoin decreased mean plasma levels of phenytoin. The rate of elimination of phenytoin decreased by approximately 80% that of the control and it may be clinically relevant in actual clinical practice where a decrease in $t_{1/2el}$ and AUC_{0-24} would lead to decreased duration of therapeutic effect of phenytoin due to rapid elimination as well as decreased oral bioavailability. In such a situation, the dose of phenytoin will have to be increased to maintain adequate control of seizures. This findings suggest that the increased metabolism and decreased bioavailability of phenytoin when aceclofenac will be given alongwith phenytoin.

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 dosage is increased (Aronson *et al.*, 1996).

It has been found that there is significant increase in rate of elimination of phenytoin with aceclofenac. Aceclofenac and phenytoin both get extensively metabolized by CYP2C9 (Nation *et al.*, 1990). Presently there are no data available on index literature about the pharmacokinetic interaction of aceclofenac with other drugs. Only theoretical knowledge is not sufficient to comment on any interaction. The reasons about the alteration in metabolism of the phenytoin are not clear. Since it has also been not found that aceclofenac is either an inducer or inhibitor of CYP2C9. So, it is too early to comment on the clinical significance of this interaction but further studies are required to comment on it because significant results have been obtained from this study.

In conclusion, aceclofenac alter the pharmacokinetics of phenytoin to a significant level. This interaction has not been reported before. Due to ethical constraints, we have included minimum number of animals as per ethical guidelines. However, the findings of this study may prove

to be important in clinical settings. 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 patient on chronic therapy for epilepsy who requires aceclofenac as a concomitant treatment for the management of chronic arthritic or inflammatory conditions.

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