Effect of 'Mentat' on the pharmacokinetics of single and multiple doses of phenytoin in rabbits

Garg SK, Islam AS, Kumar N, Sehgal M, Bhargava VK
Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.

ABSTRACT
The effect of 'Mentat', a herbal preparation, was studied on pharmacokinetics of single and multiple doses of phenytoin in rabbits. No significant effect was found after single oral dose of 'Mentat' on single dose kinetics of phenytoin. However, 'Mentat' administration for 7 days increased the steady state kinetic parameters. Peak plasma phenytoin concentration, area under the implant plasma concentration and elimination half life were significantly increased and t-max was significantly reduced, indicating the suppression of phenytoin metabolism by 'Mentat'.

INTRODUCTION
Mentat (THE Himalaya Drug Company, Bangalore), a herbal preparation containing extracts of twenty four herbs is being recommended to correct memory disturbances, learning and language disability, enuresis, anxiety, depression and neurosis. One of the important constituents of this preparation Centella asiatica is claimed to be useful as a nerve tonic and as an antiepileptic, while the other one Withania somnifera (Ashwagandha) is a CNS depressant. Phenytoin, the commonly prescribed antiepileptic drug has a narrow therapeutic range and a change in bioavailability with other drugs administered concomitantly may lead to toxic effects or therapeutic failure. During the course of antiepileptic therapy with phenytoin, patient may take indigenous preparation like Mentat. No attempt has been made to explore potential interaction between Mentat and phenytoin. Hence the present study was designed to evaluate the effect of Mentat on single and multiple dose pharmacokinetics of phenytoin in rabbits.

MATERIAL AND METHODS
Animals: The study was carried out in healthy male rabbits weighing between 1.5 to 2.5 kg. The animals were kept in isolation for atleast three weeks prior to experimentation and were maintained in standard animal house condition of 12 hour day-night cycle with the room temperature of 25 ± 2°C. They were allowed to take water ad-libitum and standard pellets diet (Him Agro Industries) once daily at 1300 hours.

Protocol: The study was divided into two phases. The first phase study was done to evaluate effect of Mentat on single dose kinetics of phenytoin in eight healthy male rabbits using randomized cross over design. After overnight fast, each rabbit was given phenytoin at a dose of 60 mg/kg, p.o. alongwith 3 ml/kg of water at 0800 hour using a soft orogastric tube. The dosage of drugs were calculated based on surface area of rabbits in comparison to human dose. One millilitre blood samples were collected at 0, 0.5, 1, 2, 4, 6, 9, 12 and 24 hours after drug administration from marginal ear vein in heparinized tubes. The plasma was separated and stored at -20°C until assayed for phenytoin. After a washout period of 7 days, the rabbits were given 60 mg/kg of phenytoin alongwith 3 ml/kg of Mentat syrup p.o. One millilitre blood samples were taken at similar time intervals as described above.
The second phase study was carried out in another group of 8 healthy rabbits to explore effect of Mentat on the kinetics of multiple dose plasma phenytoin concentrations. After overnight fast, the rabbits were given 60 mg/kg of phenytoin alongwith 3 ml/kg of water, p.o once daily at 0800 hour for 7 days. On day 7, blood samples of 1 ml each were collected at 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hours after drug treatment in heparinized tubes. Plasma was separated and kept at -20°C until analysis could be performed. From day 8 to 14 the rabbits were treated with phenytoin (60 mg/kg) alongwith Mentat syrup (3 ml/kg) p.o. once daily. On day 14, blood sampling was performed at similar time intervals as described above.

**Analysis:** Phenytoin concentrations in plasma samples were estimated by HPLC technique which was standardized in our laboratory and the intra-assay and inter-assay coefficient of variation was 3.7% and 4.6% respectively.

**Pharmacokinetic Parameters:** Peak plasma phenytoin concentration ($C_{\text{max}}$) and time to reach peak concentration ($t_{\text{max}}$) for single dose study, and steady state peak and trough plasma phenytoin concentration ($C_{\text{max}}$ and $C_{\text{min}}$) and the time to reach $C_{\text{max}}$ were calculated from actual plasma data of each rabbit. The area under the time plasma concentration (AUCo-$t$) of phenytoin was calculated by trapezoidal rule, the AUCt-$t$ was calculated by dividing the last sample plasma concentration of drug by elimination rate constant ($K_e$). AUCo-$t$ is the sum of AUCo-$t$ and AUCt-$t$. Absorption rate constant ($K_a$) and elimination rate constant ($K_e$) of phenytoin were calculated by residual method and by least square regression analysis respectively. Absorption half life ($t_{1/2a}$) and elimination half life ($t_{1/2e}$) were calculated by the formulas: $t_{1/2a} = 0.693/K_a$ and $t_{1/2e} = 0.693/K_e$. Student's paired `t' test was utilized to find level of significance of differences between pharmacokinetic data of phenytoin before and during co-administration of Mentat. $p< 0.05$ was taken to be statistically significant.

**RESULTS**

**Effect of Mentat on Single Dose Kinetics of Phenytoin:** Results of the pharmacokinetic parameters are summarized in [Table I]. No significant difference ($p>0.05$) was found between pharmacokinetic parameters of single dose phenytoin given with water or Mentat. [Figure 1] shows the effect of single dose Mentat on plasma phenytoin concentrations (Mean + SEM) at different time intervals of eight rabbits. Phenytoin concentrations co-administered with Mentat were found to be elevated in the absorption phase and suppressed in the elimination phase in comparison with phenytoin concentrations when co-administered with water. The differences were not significant ($p>0.05$) statistically. Phenytoin could be detected in 24 hours samples of all the animals after single dose administration.

**Effect of Mentat on Multiple Dose Kinetics of Phenytoin:** [Table 2] demonstrates the pharmacokinetic parameters of phenytoin before and during concomitant administration of Mentat for 7 days. Steady state peak plasma concentration ($C_{\text{max}}$), area under the time plasma concentration (AUC0-24 and AUCo-$t$) and elimination half life ($t_{1/2e}$) of phenytoin were significantly increased and time to reach the steady state peak concentration ($t_{\text{max}}$) was significantly decreased following 7 days of co-administration of Mentat. Steady state through plasma phenytoin concentration was also increased following co-administration of Mentat in comparison with that of phenytoin given with water.

[Figure 2] depicts the effect of Mentat treatment for 7 days on steady state phenytoin concentration (Mean + SEM) of 8 rabbits at different time intervals. Mentat administration increases plasma phenytoin concentrations throughout the time period under study i.e. from 0
to 24 hours after drug administration. The changes in plasma phenytoin concentration were statistically significant at time intervals 2, 3, 4, 5, 6, and 12 hours after Mentat administration.

**DISCUSSION**

Results of the present work have shown that single dose phenytoin kinetics is not affected significantly by co-administration of Mentat. In multiple dose study, when phenytoin was given with Mentat for 7 days, there was significant increase in AUC0-24, t1/2e (p<0.05) and significant decrease in steady state t\(_{\text{max}}\) (p<0.05) of phenytoin as compared to control values. Phenytoin is eliminated from the body by hepatic metabolism principally by the process of hydroxylation\(^6\). Functional status of hepatic microsomal cytochrome P450 isozyme(s) is crucial in the process of phenytoin elimination\(^9\). The increase in plasma phenytoin concentration with increase in elimination half life as has been shown in the present study indicates decreased metabolism of phenytoin when co-administered with Mentat. A recent report from this laboratory\(^10\) did not show significant difference in plasma concentrations after 7 and 14 days of phenytoin administration in healthy male rabbits. So the decrease in phenytoin metabolism as is evidenced from the present work could not be due to saturation of metabolizing isozyme(s) or auto-inhibition i.e. inhibition of the isozyme(s) by phenytoin metabolites. Hence hepatic cytochrome P450 isozyme(s) inhibition by Mentat is strongly suggestive as the underlying mechanism. Though results of the present study cannot be extrapolated to humans, it warrants clinical investigation to confirm the present findings.

**ACKNOWLEDGEMENT**

One of the authors (AFM S Islam) was supported by a fellowship from World Health Organization, Geneva (Switzerland).