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A STUDY ON THE CHRONOPHARMACOKINETICS OF THEOPHYLLINE IN RABBITS

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Abstract : The Chronopharmacokinetics of a single oral dose of theophylline was studied in rabbits. It was observed that morning (06.00 hr) dosing was characterized by significantly low rate of absorption (t1/2a and Tmax) but higher extent of absorption ($AUC_{0-\infty}$) compared to that after nocturnal dosing (22.00 hr). The plasma half life (t1/2el) was significantly less at night compared to that in daytime. The data may have considerable clinical relevance.

Key words : chronopharmacokinetics theophylline rabbits

INTRODUCTION

It has been well documented that the pharmacokinetic parameters (1,2), the therapeutic efficacy (3) and the toxicity (4) of several drugs vary according to the time of drug administration. Although such a study has been made for sustained release theophylline (5), no study related to the influence of diurnal rhythm on orally administered theophylline kinetics in animals is available. The present study was designed to investigate the chronopharmacokinetics of theophylline in rabbits since theophylline is prescribed widely in India and is a drug with narrow therapeutic effective range (8-20 μg/ml) in the treatment of bronchial asthma.

METHODS

The study was done in 8 healthy adult male rabbits (1.5 to 2.5 kg). The animals were given food (Hindustan Lever pellet diet) once a day at 14.00 hr daily except on the study days when food was given 12 hr preceeding and 12 hr after the drug administration. They had free access to water. A constant day-night cycle was maintained (light phase from 06.00 hr to 18.00 hr and dark phase from 18.00 hr to 06.00 hr). The temperature was maintained at 23±2°C throughout the day-night cycle.

A cross-over single dose chronopharmacokinetic study was undertaken with a washout period of 10 days between each two study days. Theophylline was administered orally at a dose of 10 mg/kg as aminophylline solution. Pilot studies had revealed that this dose showed the mean peak plasma concentration within the human therapeutic range. The drug was given either at 06.00 hr, at 14.00 hr or at 22.00 hr. On each occassion, the drug was given twelve hours after the last feed.

Venous blood samples (0.5 ml each) were drawn from the marginal ear vein through a heparinized cannula at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6 and 8 hr after drug administration. The samples were immediately centrifuged at 3000 rpm for 15 min, the plasma was separated, and frozen at-20°C till assayed for theophylline, using reversed phase high performance liquid chromatography technique (6) consisting of a C-18 ODS column with particle size of 5 μm. The mobile phase consisted of acetonitrile: 0.01 M sodium acetate (pH 5.0) in 10:90 v/v ratio. The flow rate was maintained at 1.2 ml/min

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and theophylline was detected by using UV detector at 273 nm. The retention time of theophylline under these conditions ranged from 5.9 to 6.1 min. The sensitivity of the method was 0.2 μg/ml and the precision as determined by intra-assay coefficient of variation was 6.78% and inter-assay coefficient of variation was 7.72%.

Pharmacokinetic calculations: The plasma concentration-time data were analysed using an one compartment open model. The following pharmacokinetic parameters were calculated: (a) Cmax (Peak plasma concentration); (b) Tmax (Time of peak plasma concetration) i.e. time at which Cmax occured; (c) t1/2a (absorption half life) calculated by the residual methods; (d) t1/2el (elimination half life) calculated by least square regression analysis of the monoexponential declining line of the plasma concentration-time curve; (e) $AUC_{0-\infty}$(Area under the plasma concentration-time curve) calculated using the trapezoidal rule and (f) $AUC_{8-\infty}$ ($AUC_{8-\infty}$ = Ct/Kel, where Ct is the theophylline concentration at the last sampling time and Kel is the elimination rate constant. Finally, $AUC_{0-\infty}$ for each rabbit was determined by the addition of AUC_{0-8} and $AUC_{8-\infty}$.

Statistics: Analysis of variance was used to compare the values (Mean ± SEM) of a given parameter. If the 'F' value was found to be significant then student's 't' test was employed for inter-group comparision. $P<0.05$ was considered statistically significant.

RESULTS

Fig. 1 shows the plasma theophylline concentration-time curve for a 8 hr period after oral dosing (10 mg/kg) using three temporally different schedules (i.e. 06.00 hr, 14.00 hr and 22.00 hr). The results show wide inter individual variation in the attained plasma concetration at any given point of time of sampling. Each curve shows a rapid rise in plasma theophylline levels to attain a peak followed by a gradual fall. The plasma theophylline levels were significantly higher for the 06.00 hr drug administration group at 2,4,6 and 8 hr sampling periods compared to that after 22.00 hr administration while no

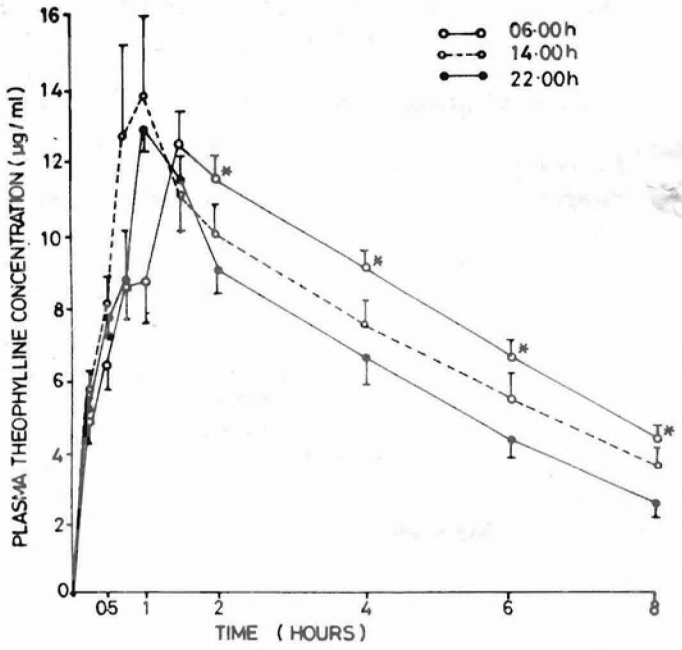

Fig. 1: Plasma theophylline concentrations at different time intervals after single oral dose administration to eight rabbits under three different time schedules i.e. 06.00 hr, 14.00 hr and 22.00 hr. Each point represents Mean ± SEM from eight observations.

*P<0.05 between the 06.00 hr and 22.00 hr.

TABLE : Effect of circadian variation on single oral dose theophylline bioavailability parameter in rabbits.

S. No.	Groups	Cmax (µg/ml)	Tmax (hr)	t1/2a (hr)	t1/2el (hr)	$AUC_{0-\infty}$ µg/ml.hr)
1.	06.00 hr	12.47±0.94	1.69±0.13	0.54±0.08	4.29±0.23	92.73±6.72
2.	14.00 hr	13.79±2.15	1.03±0.11+	0.39±0.11+	4.07±0.42	81.78±7.67
3.	22.00 hr	12.94±0.58	1.13±0.08*	0.34±0.05*	3.03±0.25*	63.52±6.55*

Values are Mean±SEM (n=8)
+2 Significantly different from 1 at $P < 0.05$
*3 Significantly different from 1 at $P < 0.05$

significant difference was observed in the plasma concentration at any point of time between the 14.00 hr and 22.00 hr groups and between 06.00 hr and 14.00 hr groups.

Table I shows the related pharmacokinetic parameters. Tmax was significantly higher in the 06.00 hr group as compared to both the 14.00 hr and 22.00 hr groups. It should however, be emphasized that more precise estimate of Tmax could be achieved by more frequent sampling. tl/2a was significantly higher in the 06.00 hr group compared to both the 14.00 hr and 22.00 hr groups. t1/2el of theophylline was significantly less in 22.00 hr group as compared to that of 06.00 hr group but there was no significant difference between the 06.00 hr and 14.00 hr and also between the 14.00 hr and 22.00 hr groups.

$AUC_{0-\infty}$ was significantly greater with 06.00 hr group as compared to that of the 22.00 hr group while no significant difference was observed between the 06.00 hr and 14.00 hr and also between the 14.00 hr and 22.00 hr groups. No significant difference was observed in the peak plasma concentration (Cmax) amongst the three groups.

DISCUSSION

Theophylline is a widely prescribed bronchodilator with a relatively narrow therapeutic range. Plasma concentration of theophylline correlates with its therapeutic effects and adverse effects. However, the attained plasma concentration after a given dose shows interindividual variation and itself Is influenced by several determinants like age, disease and smoking habit (7,8,9). Diurnal rhythm also may have an influence but attention has not been paid to study it. Oral bioavailability of sustained release theophylline is not different when the drug was given in the morning and in the evening (10). Neither there was difference in the iv pharmacokinetic profile of single dose of aminophylline given at 09.00 hr and 21.00 hr (11). Jackson et al (12) reported significantly higher Tmax and lower AUC_{0-12} after 23.00 hr dosing compared to that of 11.00 hr dosing of sustained release theophylline given orally.

In the present study it was observed that the morning time dosing had significantly reduced rate of absorption of orally administered theophylline compared to that after afternoon (14.00 hr) and night time (22.00 hr) dosing. No significant difference in the peak concentration was observed although the extent of absorption was found to be significantly lower at night compared to that after morning time dosing. Differences between the present results and the previously reported results (see above) could be related to the formulation difference (solution versus sustained release), route of administration of drug (oral versus intravenous), differences in the time of drug administration and species difference (rabbit versus human being). The exact reason for slower absorption of theophylline in the morning is unknown. It cound be related to diurnal changes in gastric emptying, gastrointestinal motility and splanchnic circulation.

However, the lesser $AUC_{0-\infty}$ at night was due to significantly lower plasma concentration of theophylline at night compared to that after morning at each time during the drug elimination phase. Half life of theophylline was also found to be significantly less at night compared to that in the morning. Faster elimination of theophylline at night might lead to subtherapeutic concentration of theophylline during the late night hours resulting in the loss of antiasthmatic effect of theophylline. This observation is of significant clinical importance necessitating careful dosage adjustment at night or replacement of conventional theophylline by sustained release ones at night in order to maintain adequate plasma concentration throughout the night. The precise reason for faster metabolism of theophylline at night is not clearly known. However, this could be due to the influence of circadian rhythm on the liver drug metabolizing activity. *In vitro* studies suggest such influence in case of some chemicals (13).

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