Circadian Rhythm in Theophylline Disposition during a Constant-Rate Intravenous Infusion of Aminophylline in the Dog

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Abstract □ The disposition of theophylline in three dogs was determined during a 48-h constant-rate intravenous infusion of aminophylline. A systematic fluctuation in serum theophylline concentrations was observed over a 24-h period, which appeared to be characteristic of a circadian rhythm. Neither assay variability nor fluctuations in the infusion pump rate could account for the observed variations in the serum concentrations. It was concluded that the changes in the theophylline concentrations were the result of a circadian rhythm in theophylline disposition.

Few attempts have been made to obtain frequent drug serum concentrations at steady state, during a constant-rate intravenous infusion of theophylline for periods equal to or greater than 24 h.[1,2] Results from those studies have been inconclusive in detecting a circadian rhythm in the clearance of theophylline in humans. However, other studies have reported differences in theophylline elimination half-life in subjects administered aminophylline intravenously in the morning as compared with the evening.[3-6] The purpose of the present study was to determine if an apparent circadian rhythm in disposition could be seen during constant-rate intravenous infusions of theophylline. Dogs were used in the study, since it was relatively easy to have strict control over their environment.

During a constant-rate intravenous drug infusion, serum concentrations increase and eventually plateau when the rate of drug input is equal to the rate of drug elimination. Approximately 88% of steady-state equilibrium should be reached after three drug half-lives have elapsed from the start of the infusion. The half-life of theophylline in the dog has been found to be in the range of 4.5 to 8 h, based on preliminary investigations in this laboratory. Thus, these dogs should be at least at 88% of steady state after 24 h, and at 98% after 48 h of a constant-rate infusion. Any change in the clearance or the apparent volume of distribution of a drug at steady state should be inversely related to changes in serum concentration. Thus, any rhythm in disposition should be reflected by a rhythm in the observed serum concentrations.

Experimental Section

A portable infusion pump (AutoSyringe, model AS*2F) was used to provide each dog with a constant-rate infusion for a period up to 49.5 h. The pump rate was validated with a 20-mL syringe (Becton Dickinson) at a rate of ~ 0.41 mL/h. Samples for the pump validation were infused into preweighed vials which were fitted with a rubber septum, and vented during the individual collection periods. Each collection period was ~4 h and the total time of infusion was ~49 h. The individual vials were reweighed at the end of the infusion to determine the amount of solution pumped per collection period.

During actual experiments, the loaded syringe pump was placed in a pocket of a dog jacket (Alice King Chatham Medical Arts). The pump drive mechanism and controls were protected by a housing constructed of aluminum sheet metal and were counter-balanced by an equal weight of ~1.5 kg, with similar bulk, on the lateral sides of the dog. An infusion line from the pump was connected to an 8 in- (20.3-cm) teflon catheter placed in the jugular vein of the dog.

An aminophylline solution for infusion was manufactured from 25.1 g of aminophylline (Sigma), 2.21 g of disodium ethylene tetraacetate (Fisher Scientific Company), 3.5 mL of ethylene diamine (Mallinckrodt, Inc.), and enough reverse osmosis water to produce 250 mL of solution. The final solution had a pH of 9.1 at 25 °C. This solution was dispensed into sterile 30-mL vials under aseptic conditions via a sterile Burron Multi-Add Dispensing System (30 mL; Burron Medical Inc.), through a sterile 0.2-μM Acrodisc filter (Gelman Sciences). No attempt was made to adjust osmolality since the volume of the solution to be infused was <0.4 mL/h, and the solution was being infused into a relatively large vein.

To prevent catheter clotting during infusion, 1.5 mL of heparin (1000 units/mL) was added to the aminophylline solution to give an approximate concentration of 50 units/mL. This solution was determined by HPLC analysis to contain an equivalent of 84 mg/mL of theophylline. The aminophylline infusion rates ranged from 18.7 to 33.9 mg of theophylline per hour. The rates were calculated to give an average concentration of theophylline at steady state between 10 and 20 μg/mL, based on previous determinations of the theophylline elimination half-life in these dogs. Serum samples were obtained through a 2-in (5.08-cm) teflon catheter placed and maintained in the left cephalic vein of the dog. Approximately 7 mL of blood was taken at ~3-h intervals 21 to 48 h after starting an infusion in the morning, and 10 to 49 h after starting an infusion in the evening.

The assay used to determine theophylline serum concentrations was adapted from a reversed-phase liquid chromatography method described by Farrish and Wargin.[7] The equipment (Waters Associates) consisted of a variable wavelength detector (model 481) set at 0.05 AUFS and 280 nm, an auto-injector (WISP model 410B), and a pump (model 510) set at 1.5 mL/min. The detector output was monitored with a recorder (Fisher Recordall Series 5000) at a chart speed of 0.25 cm/min. The column was a 150 mm × 3.9 mm (internal diameter) μBondaPak C-18 column (Waters Associates). The mobile phase consisted of 5% acetonitrile, 0.0025 M tetrabutylammonium phosphate, and 0.02 M monobasic sodium phosphate. The pH was adjusted to 4.1 with concentrated phosphoric acid. Standard solutions of theophylline (Sigma Chemical Company) and beta-hydroxyethyl-theophylline (Sigma Chemical) were prepared in 1% methanol with deionized water at a concentration of 100 μg/mL. Standard curves were prepared on a daily basis at concentrations of 0.5, 1.0, 5.0, 10.0, and 20.0 μg/mL of theophylline. Samples were prepared with 0.25 mL of drug-free dog serum, 0.25 mL of internal standard solution (20 μg/mL), and 0.25 mL of theophylline solution. Each fortified serum sample was extracted in a 15-mL screw cap tube using 5.0 mL of 3% isopropanol in chloroform, with horizontal shaking for 20 min. The samples were centrifuged, and the aqueous top layer was aspirated to waste. The bottom layer was transferred to a silanized conical test tube and dried at 35 °C under nitrogen. Dried samples were reconstituted with 100 μL of mobile phase by vortexing for ~15 s, and 20 μL was injected onto the column.

Three female mongrel dogs, ~20 kg each, were utilized in the

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study. The dogs were housed at the University Animal Resources facility with free access to food and water. They were maintained on a lighting schedule of "lights on" from 6 a.m. to 6 p.m. (06:00 to 18:00) and "lights off" from 6 p.m. to 6 a.m. (18:00 to 06:00). The dogs were fed Purina Lab Canine Diet #5006. Temperature was maintained within a range of 69 to 79 °F. Illumination throughout the dog housing was provided by 4-ft (1.22-m) flourescent lights (Sylvania Super Saver, Cool White, F40/CW/RS/SS, 34 W), which yielded an average illumination of ~50 foot-candles and ranged from 4 to 194 foot-candles at the shoulder level of the dogs. There were no windows in the dog housing. The infusion experiments were conducted during the months of July and August, 1987.

Results and Discussion

A circadian rhythm is exemplified by a systematic fluctuation in a measured value over a period of time of ~24 h. As shown in Figure 1, there appeared to be an obvious rhythmic fluctuation in the serum theophylline concentrations measured in each dog during a prolonged intravenous infusion. Peaks in the serum drug concentrations were observed to occur at 24 to 30 h after starting the morning infusions, while the troughs in the serum drug concentrations were observed to occur at 36 to 42 h after initiating the morning infusions, as shown in Figure 1a–1c. This pattern was reversed for the data set obtained in Dog 3, after starting the intravenous infusion in the evening. As shown in Figure 1d, the peak and trough occurred at ~39 and 24 h, respectively, after starting the evening infusion. The peak and trough for the individual serum drug–time profiles were separated by ~12 h in each dog, which is consistent with a circadian rhythm.

Two experimentally introduced artifacts could have been responsible for the observed fluctuation in the serum concentrations. One possibility was that the infusion pump did not provide a constant input of drug. However, as summarized in Table I, the amount of solution delivered per hour was quite constant, with a percent coefficient of variation (%CV) of only 1.2%. The first sample in the series was determined to be an outlier using Dixon's test for extreme values.[8] This low value was attributed to a lack of constant flow immediately after starting the infusion. A second source of error could have been the method employed for the serum assays. Ten standard curves were prepared in duplicate on 10 different days. The back-calculated concentrations for the 10-μg/mL standard ranged from 9.5 to 10.3 μg/mL, with a mean of 10.0 μg/mL and a %CV of 2.4%. Similarly, the back-calculated values for the 20-μg/mL standard ranged from 19.9 to 20.6 μg/mL, with a mean of 20.1 μg/mL and a %CV of 1.5%. Further, the small error associated with the assay appeared to be random. Thus, it was concluded that the magnitude and systematic

Table I—AutoSyringe Pump Rate Validation

Elapsed Time, h	Rate, g/h
4.15	0.3700[a]
7.35	0.4061
11.57	0.4208
15.68	0.4120
21.12	0.4180
25.05	0.4121
29.05	0.4178
33.95	0.4135
37.58	0.4204
40.82	0.4220
49.05	0.4142
Mean	0.4157
SD	0.0050
% CV	1.1970

[a] Number was determined to be an outlier (ref 8).

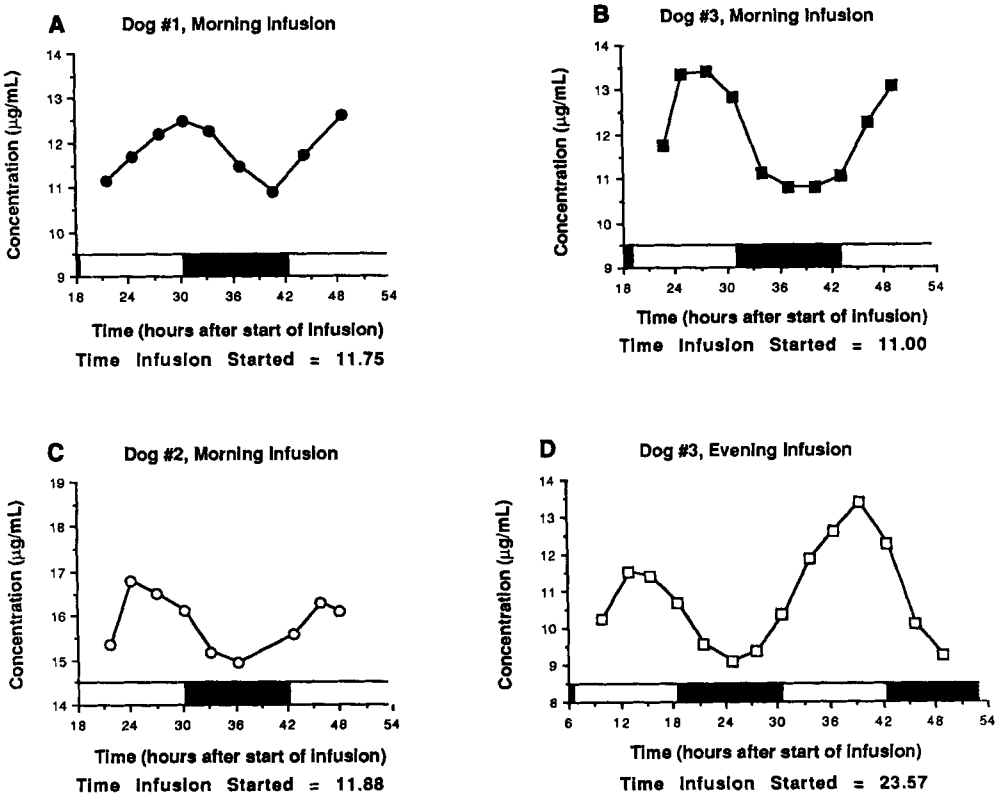

Figure 1—*Serum theophylline concentrations in three dogs during a constant-rate infusion of aminophylline approaching steady state. The light and dark shaded bar indicates the times of lights on and lights off, respectively.*

nature of the changes seen in the serum theophylline concentrations over the sampling period in each dog, as summarized in Table II, were not the result of either fluctuations in the infusion pump or variability in the theophylline assay.

A comparison of the steady-state infusion profiles revealed that the apparent circadian rhythm responsible for the changes in the serum concentrations seen in each dog was approximately synchronized (Figure 2). The peaks and troughs of the serum concentrations resulting from all infusions occurred at approximately the same time of day. Dog no. 3 was restudied with an infusion that was started in the evening, ~12 h out of phase with the previous infusion experiment that was started in the morning. The resulting circadian variation in serum concentration was observed to be ~12 h out of phase (Figure 1d) with the results from the infusion started in the morning (Figure 1b), when comparing serum drug concentrations versus hours after start of infusion. It was observed that the apparent circadian rhythm responsible for changes in serum concentrations during the steady-state constant-rate infusions resulted in maximum serum concentrations during the period from 12 noon to 6 p.m. (12:00 to 18:00), while the troughs were observed during the period from 12 midnight to 6 a.m. (24:00 to 06:00).

Some previous investigators have not observed a circadian rhythm effect for theophylline disposition during a constant-rate infusion of aminophylline administered to hospitalized asthmatic patients,[1,2,9] but no indication was given as to prior control of the activity–rest schedules of the patients used in the studies. In the study conducted by Uematsu et al.,[2] no consistent trend was observed for changes in individual serum concentration profiles during a constant-rate intravenous infusion at steady state over a 24-h period. Timing of standard meals was controlled in the study; however, additional food intake and physical activities were not. In the study conducted by Rogers et al.,[1] a constant-rate infusion of aminophylline was approximated by administering an infusion each hour during the 24-h period of the infusion study. This type of protocol could be susceptible to errors in dosing.

Table II—Serum Theophylline Concentration Range during a Constant-Rate Intravenous Infusion

Dog No.	Time	Range, μg/mL	Range Difference, μg/mL
1	Morning	10.9–12.5	1.6
2	Morning	14.9–16.8	1.9
3	Morning	10.8–13.4	2.6
3	Evening	9.1–13.4	4.3

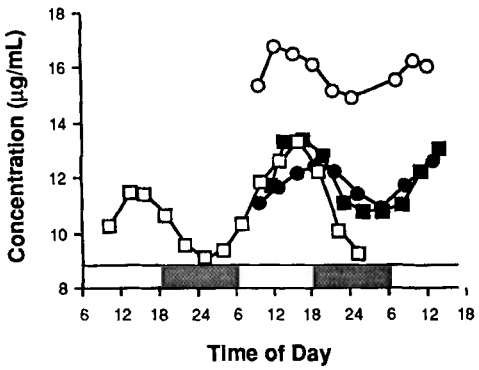

Figure 2—*Serum theophylline concentrations in three dogs during a constant-rate infusion of aminophylline approaching steady state, with time expressed as time of day. Key: Dog 1 (●); Dog 2 (○); Dog 3, a.m. infusion (■); Dog 3, p.m. infusion (□).*

In addition, the results of serum concentrations obtained during a 24-h infusion period were only reported as a mean and standard error for the eight patients studied. Thus, if the time of peak and trough serum concentrations were out of phase in different patients, examination of only mean serum concentration–time profiles could obscure any apparent circadian rhythm effect. A study by Coulthard et al.[9] utilized five asthmatic children to evaluate a potential circadian clearance of theophylline 12 to 36 h after starting an infusion of aminophylline. Only two concentrations were determined for each patient, from samples collected at 09:00 and 21:00. Based on the average ratio of the 09:00-to-21:00 plasma theophylline concentrations, these investigators[9] concluded there was no effect of a circadian rhythm on theophylline clearance. The accuracy and precision of pumps used in these studies were not discussed.[1,2,9]

A statistically significant difference in theophylline elimination half-life has been observed in humans after a 5-h intravenous infusion in the morning as compared with the evening.[4] The theophylline half-life was reported to be greater in the resting period (03:00 to 10:00) and shorter in the activity period (15:00 to 22:00) of diurnally active human subjects. These half-life estimations were based on a relatively short sampling period (7 h). The observed difference in clearance was only 8%. Taylor et al.[3] also found a shorter elimination half-life in healthy human subjects after a 30-min intravenous infusion in the morning (10:30 to 22:00) as opposed to dosing in the evening (22:30 to 10:00), although the difference was not statistically significant. Also, no difference in systemic clearance was observed in their study,[3] as determined from the dose divided by the area under the plasma concentration–time curve. In another study by Giacona et al.,[5] mean clearance of theophylline was found to be greater in 10 normal, healthy subjects after a 30-min intravenous infusion of aminophylline that was given in the morning as compared with the evening. Chauhan et al.[6] conducted a study in five healthy male subjects which were administered an intravenous dose of theophylline at 7 a.m. (07:00) and 7 p.m. (19:00). A significantly shorter theophylline elimination half-life was reported after the morning dose compared with the evening dose. Based on the results of these studies,[3–6] it is conceivable that the apparent changes in theophylline disposition observed in the present study may be due to changes in clearance (*CL*). Another factor which could be responsible for the observed circadian effect is a change in volume of distribution (*Vd*). For example, a cyclical increase in *Vd* could result in a cyclical decrease in plasma theophylline concentration. Jonkman et al.[4] reported a slightly higher *Vd* after dosing theophylline in the morning; however, the maximum plasma concentrations at the end of the morning and evening infusions were almost identical. In contrast, Taylor et al.[3] found a slightly higher *Vd* after an intravenous dose given in the evening compared with morning dosing. Finally, Giacona et al.[5] reported the *Vd* was not significantly different after an intravenous dose of aminophylline given in the morning and in the evening. Based on these studies,[3–5] changes in *Vd* appear to be relatively small compared with the observed changes in *CL*; however, a circadian rhythm in *Vd* cannot be discounted as being responsible for the observed changes in serum concentrations seen in the present study.

Differences in the magnitude of the apparent circadian rhythm of theophylline disposition in the present study and previous work[1–6,9] may simply reflect inherent differences between the dog and humans. Direct comparisons are difficult since individual activity–rest cycles for each human prior to and during participation in each study is not known. It would appear that a constant-rate infusion at steady state provides a useful approach to discerning the presence of a

circadian rhythm for theophylline clearance. Further, with frequent sampling (e.g., every 3 h), the apparent time of day when the clearance, or possibly the volume of distribution, reaches a maximum or minimum may be estimated from fluctuations in the serum theophylline concentration.

The results of the present study have clearly illustrated the presence of an apparent circadian rhythm in the disposition of theophylline in the dog. The dog was an excellent model since it could be maintained under controlled environmental conditions for a prolonged period of time. Further, it was possible to administer an extended constant-rate infusion and obtain multiple blood samples throughout the course of the infusion.

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