Variations in Prothrombin Time and International Normalized Ratio over 24 Hours in Warfarin-Treated Patients


Study Objective. To determine the variation of prothrombin times and international normalized ratio (INR) over 24 hours in humans.

Design. Prospective, parallel study.

Setting. University-affiliated general clinical research center.

Patients. Six patients receiving long-term warfarin therapy and six sex-matched controls.

Interventions. Warfarin was administered to the patients at 6:00 P.M.

Measurements and Main Results. Prothrombin times and INR were determined every 2 hours over 24 hours. Time of study entry, meals, and sleep cycles were controlled. A significant cosinor rhythm for prothrombin times and INR (p≤0.03) occurred in warfarin-treated patients, suggesting that diurnal variation occurs. The mean difference between the peak and trough prothrombin times was 1.8 ± 0.9 seconds (range 0.8–3 sec) with a mean change of 9.3% ± 3.7%. The peak prothrombin time and INR values occurred between 4:00 A.M. and 8:00 A.M. in five patients, and trough values between 6:00 P.M. and midnight in five. No significant cosinor rhythm was noted for controls (p>0.5).

Conclusion. Significant variations in prothrombin time and INR occurred in patients receiving warfarin therapy, with the highest values occurring in the morning and the lowest in the evening. These results may have clinical implications for patients receiving either high- or low-intensity warfarin therapy.


Time-dependent variations (circadian or diurnal) in physiologic processes and pharmacologic responses may have important clinical implications. For example, a drug’s pharmacokinetic and pharmacodynamic properties may be influenced by endogenous and exogenous rhythms, and these properties may vary depending on the time of day for a given individual. Therefore, a patient's therapy may have to be optimized according to such diurnal variations. Circadian changes in effect have been observed with a number of drugs, including corticosteroids, theophylline, and chemotherapeutic agents.1,2

Circadian or diurnal variations in the pharmacodynamics of anticoagulants have also been reported.3–5 Time-dependent alterations in both activated partial thromboplastin time (APTT) and thrombin time occurred after constant infusions of heparin.3 Animal data show that warfarin's pharmacodynamic effect on clotting
times and the vitamin K cycle has similar variations.4,5 These findings suggest that patients receiving warfarin may theoretically be at risk of having subtherapeutic or supratherapeutic anticoagulation effects depending on the time of day.

The purpose of this study was to determine warfarin's pharmacodynamic effect in humans over 24 hours as measured by prothrombin times. The results will determine if warfarin's effects have a diurnal variation, and if so, its potential clinical implication.

Methods

The study was approved by the institutional review board and was performed in the general clinical research center. Six patients receiving warfarin therapy and six sex-matched controls were enrolled in this prospective, parallel design study. Each group consisted of four men and two women. Patients receiving warfarin were stabilized with their dose (4.1 ± 2 mg) for at least 1 month before the study and had established dosing times between 5:00 and 8:00 P.M.

Patients were included if results of liver function, renal function, hematologic, and blood chemistry laboratory tests were within normal limits, and if they had no history of a bleeding episode within the last 6 months. Mean aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase values were 25 ± 2 IU/L (normal range 2–35 IU/L), 33 ± 7 IU/L (normal range 0–45 IU/L), and 157 ± 24 IU/L (normal range 60–200 IU/L), respectively. Patients were excluded if they had been taking any agents known to inhibit or induce the metabolism of warfarin or alter the protein binding of warfarin for less than 30 days before study entry. Controls were considered eligible based on history, physical examination, blood chemistry, and hematologic screen.

All participants were included only if they had an entrained sleep-wake cycle with sleeping hours occurring between midnight and 5:00 A.M. at the minimum. They were instructed to adhere to a regular sleep-wake cycle for at least 3 days before study initiation.

Participants were admitted to the research center at approximately 2:00 P.M. on their assigned study day. Peripheral venous catheters (Becton Dickinson, Sandy, UT) were placed, and blood samples (approximately 2 ml) for measuring prothrombin time and international normalized ratio (INR) were collected in vacutainer tubes containing sodium citrate every 2 hours beginning at 4:00 P.M. and continuing for 24 hours. Approximately 4 ml of blood was removed and discarded before obtaining a sample. After each blood collection the venous catheter was flushed with normal saline to help maintain patency. All samples were processed immediately. Patients receiving warfarin were administered their individual dose at 6:00 P.M. All subjects were required to be in bed with the lights out between 10:00 P.M. and midnight and were awakened at 6:45 A.M. Standard meals were provided at times 8:00 A.M., 12:00 noon, and 5:00 P.M.

Laboratory Analysis

Prothrombin times and INR were determined by the hospital's hematology laboratory using the Baxter Dade thromboplastin C test (American Dade Co., Miami, FL). The international sensitivity index value equaled 2 for the study period. The intraday and interday standard deviations within a normal prothrombin time range (11.3–13.0 sec) were 0.1 and 0.9 seconds, respectively. The intraday and interday standard deviations for prothrombin times exceeding the normal range (16.0–30 sec) were 0.2 and 2.9 seconds, respectively.

Data Analysis

Cosinor analysis was performed on the raw data to evaluate the effect of time and to determine if there was a significant rhythmic variation in the data with respect to time. A p value below 0.05 was considered statistically significant. Data are summarized as means and standard deviation where appropriate.

Results

All 12 participants completed the study. The mean age of the warfarin-treated patients was 69 ± 11 years and of controls was 34 ± 13.8 years. All patients were compliant with their warfarin regimens as assessed by questioning. The indications for warfarin therapy were cardiomyopathy (2 patients), valve replacement (1), deep vein thrombosis (1), and atrial fibrillation (2). Concurrent drugs were diltiazem, furosemide, potassium chloride, aspirin, enalapril, naproxen, prednisone, hydroxychloroquine, cisapride, famotidine, propranolol, nifedipine, and lovastatin. All patients were stabilized with these agents for at least 1 month.

The overall mean prothrombin time and INR values for the warfarin group were 20.0 ± 2.5
seconds (range 16.8–24.8 sec) and 2.6 ± 0.6 (range 1.9–3.9), respectively. The overall mean figures for the control group were 12.2 ± 0.4 seconds and 0.97 ± 0.07, respectively.

A significant cosinor rhythm was observed for prothrombin time and INR (p≤0.03) in the warfarin group, suggesting a diurnal variation. No significant cosinor rhythm was observed for the control group (p>0.5). Mean INR values over the study period for the warfarin group are shown in Figure 1. For individual INRs the mean difference between peak and trough values was 0.18 ± 0.12 (range 0–0.3) with a mean change of 10.6% ± 6.5%. The mean difference between individual peak and trough prothrombin times was 1.8 ± 0.9 seconds (range 0.8–3 sec), with a mean change of 9.3% ± 3.7%. Peak values occurred between 4:00 and 8:00 A.M. in five warfarin-treated patients and trough values between 6:00 P.M. and midnight in five.

Discussion

The results of this study, as evidenced by cosinor analysis, showed a significant diurnal variation in prothrombin times and INR values over 24 hours in patients receiving long-term warfarin therapy. The greatest pharmacodynamic effect appears to occur in the morning (4:00–8:00 A.M.) with the least effect occurring in the evening (6:00 P.M.–midnight).

No significant diurnal variation in either value was observed in the control group. This suggests that the time-dependent variations in patients receiving warfarin may be the result of different effects of the drug on the clotting cascade. This may include a time-dependent effect on warfarin's ability to inhibit vitamin K and vitamin K-epoxide reductases, which is the mechanism by which it produces its anticoagulant effect. In support of this hypothesis, a study in rats demonstrated time-dependent differences in the activity of both reductases and the ability of warfarin to inhibit them. The agent's greatest inhibitory effect in the animal study occurred at the beginning of the sleep cycle compared with the beginning of the activity cycle.

When evaluating these results and comparing them with findings in this study, it appears that the timing of maximum effect is somewhat different. Why this occurs is uncertain, but it may be due to a number of reasons including model (human vs nocturnal animal) and study design. The animal study administered only a single dose of warfarin followed 8 hours later by vitamin K administration, and it measured only two time points. In this study we had steady-state levels of warfarin and numerous measurements to evaluate, making it more likely that we would detect changes at different times.

Another potential mechanism for the diurnal variation may relate to the time of drug

![Figure 1. Mean (± SD) INR values in warfarin-treated patients and controls.](image-url)
administration and the resultant effect on the coagulation cascade. In other words, does the administration of warfarin at 6:00 P.M. cause peak effects on the inhibition of clotting factors in the morning hours despite having steady-state levels of a drug with a prolonged half-life? In addition, could diurnal variations in warfarin's pharmacokinetic properties explain the results? To determine the effect of administration time on prothrombin times, additional studies are required to compare morning and evening dosing. Pharmacokinetic studies would also be required to ensure that similar concentrations of the R and S isomers are achieved and to determine if warfarin's pharmacokinetic properties (including stereoselective metabolism) are influenced by the time of administration.

In support of our findings, another group demonstrated that heparin also influences the clotting cascade in a diurnal fashion. Specifically, significant variability in APTT values was observed during constant heparin infusion. Of interest, despite diurnal variation in anticoagulation for both heparin and warfarin, the time course and magnitude of variation are different between the agents. The degree of variability of anticoagulation is much greater after heparin than warfarin (50% with APTT after heparin vs 10.6% with INR after warfarin). In addition, the maximum effect is highest in the evening with heparin therapy, whereas peak effect occurred in the morning during warfarin therapy. The reasons for these differences are not certain, but may relate to how the drugs are administered or how each one interferes with the clotting cascade.

Overall, the clinical significance of this study for most patients is probably minimal. However, in those receiving either low- or high-intensity warfarin therapy the degree of variation in prothrombin times may be important. For example, in patients receiving low-intensity therapy, the optimum time to measure a single prothrombin time may be during the evening hours when the prothrombin times are lowest. If a subtherapeutic effect is going to occur, this is when it is most likely. In contrast, in patients receiving high-intensity warfarin therapy, the optimum time to measure prothrombin time may be during the morning when the values are highest. If a supratherapeutic effect is going to occur, this is when it is most likely.

This study had a number of limitations, including evaluating only one administration time. Another limitation was not stratifying patients or controls according to intensity level of warfarin dosing, disease state, or age. Potentially, assay variability may have contributed to our significant findings, since the higher the value the greater the variability. Alternatively, variability may be inherent in the diurnal variation occurring in a patient, and not the assay.

Although clinical algorithms designed to minimize toxicity and maximize efficacy frequently target a given prothrombin time or INR, we have no data to demonstrate that diurnal changes are important. Furthermore, we have no data to demonstrate that by adjusting the dosage of warfarin according to a patient's diurnal variation in prothrombin times, morbidity and mortality will be reduced. However, since we often target a given prothrombin time or INR value, it may be theorized that time-related variations in warfarin's effect may influence monitoring and optimum therapy.

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References