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Impact of telavancin on prothrombin time and activated partial thromboplastin time as determined using point-of-care coagulometers

Michael P. Ero · Nathaniel R. Harvey · Jack L. Harbert · James W. Janc · Kay H. Chin · Steven L. Barriere

Abstract Telavancin is approved in the United States, Canada, and Europe (At the time of submission, the telavancin European marketing authorization for nosocomial pneumonia was suspended until Theravance provides evidence of a new European Medicines Agency approved supplier) as an antibiotic to treat certain Gram-positive bacterial skin infections. Telavancin has been shown to prolong plasmatic prothrombin (PT) and activated partial thromboplastin (aPTT) clotting times in clinical diagnostic lab-based assays. In this study, we evaluated the potential for telavancin to prolong whole blood PT/International Normalized Ratio (INR) and aPTT tests on point-of-care (POC) instruments. Whole blood collected from 8 healthy subjects was supplemented with telavancin to final concentrations of 0, 10, 20, and 100 μg/ml. Final concentrations were selected to match trough, twice trough, and peak plasma levels following the approved 10 mg/kg dose. Four widely employed POC coagulation instruments were chosen to be representative of the POC platforms currently in use.. These systems were the Roche Coaguchek XS, the Abbott iSTAT, the ITC Hemochron SIG+, and the Alere INRatio2 POC devices. The PT/INR measured by the Coaguchek XS showed the greatest sensitivity to the presence of telavancin. The PT/INR measured by the Hemochron SIG+ and iSTAT were sensitive to telavancin but to a lesser extent. The INRatio2 was the least sensitive to the presence of telavancin when testing the whole blood PT/INR. Only the Hemochron SIG+ device was capable of measuring aPTT and showed a concentration-dependent increase in aPTT. This study supports the current recommendation that PT and aPTT monitoring be conducted immediately to the next dose of telavancin when coagulation parameters are tested using POC instrumentation.

Keywords PT/INR · Point-of-care · Telavancin · Prothrombin time · Activated partial thromboplastin time

Introduction

Telavancin is a lipoglycopeptide antibiotic approved in the United States and Canada for the treatment of patients with complicated skin and skin structure infections due to Gram-positive pathogens, and in the United States and Europe[1] for the treatment of hospital-acquired pneumonia (including ventilator-associated pneumonia) due to susceptible isolates of *Staphylococcus aureus* (methicillin-resistant strains, MRSA only in Europe), when alternative medicines are unsuitable. Telavancin is active against a broad range of Gram-positive pathogens, including MRSA. In phase 1 studies, transient prolongations of prothrombin time (PT) and activated partial thromboplastin time (aPTT) were observed [1]. It was postulated that telavancin may have interfered with the exogenous phospholipids added to these assays, leading to falsely prolonged readings. Phase 2 and 3 clinical trials have not indicated an increased risk of

M. P. Ero (✉) · N. R. Harvey · J. L. Harbert
Specialized Coagulation Laboratory, Machaon Diagnostics, Inc., Sherrick Research Building, 3023 Summit Street, Oakland, CA 94609, USA
e-mail: mike.ero@machaondiagnostics.com

J. W. Janc · K. H. Chin · S. L. Barriere
Theravance, Inc., South San Francisco, CA, USA

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coagulation abnormalities in patients treated with once-daily telavancin at a dose of 10 mg/kg [2–4]. Hence, the effects of telavancin on PT and aPTT results seem to be due to interference with these laboratory tests. Current prescribing guidelines suggest that blood be sampled from patients receiving telavancin immediately preceding the next dose.

A recent study evaluated the effect of in vitro supplementation of telavancin on a variety of PT and aPTT assays that are in current use [5]. In this study, telavancin was observed to prolong both PT and aPTT in a concentration-dependent manner. Telavancin concentrations comparable to expected peak drug levels produced prolongation of clotting time with all 16 PT reagents tested. At drug concentrations comparable to expected trough levels, PT prolongation was variable, but assays utilizing reagents containing recombinant tissue factor were most affected. Trough levels of telavancin did not significantly impact aPTT assays, but all 7 reagents evaluated were impacted by higher drug concentrations. aPTT reagents containing silica activators appeared to be particularly sensitive.

Point-of-care (POC) coagulation testing is in widespread use. A variety of assay formats and instrument suppliers are currently available. Compared with traditional venous blood collection and testing, POC testing is expected to improve warfarin therapy due to better turn-around time and patient compliance with monitoring due to the preference for finger-stick sampling. This study evaluated the effects of telavancin supplementation measured ex vivo on four widely used PT POC coagulometers, with one instrument also capable of measuring aPTT.

Study design

Test agent

Telavancin was acquired by Machaon Diagnostics, Inc. in the form of pharmaceutical grade VIBATIV® (telavancin) 250 mg vials, lot number 2029222; expiration date Jan 2015. The vehicle control consisted of the excipients hydroxypropyl-beta-cyclodextrin (HBC) and mannitol. Working solutions were stored at 2–8 °C and were used within 72 h of preparation.

Device selection

POC devices were selected for this study based on the following criteria: (1) selected devices combined will have performed >50 % of the total number of tests reported by the 2009 College of American Pathologists proficiency testing survey data, (2) had high sales volumes, and (3) had either Clinical Laboratory Improvement Amendment (CLIA)-waived or moderate complexity ratings [6]. These selection criteria, taken together, are likely to represent >50 % of the total number of PT/International Normalized Ratio (INR) tests performed in the POC clinical setting in the United States in a given year.

Prothrombin time and activated partial thromboplastin time assays

PT assays were performed using the Abbott iSTAT POC Meter (Abbott, Abbott Park, IL, USA), Hemosense INRatio2 POC Meter (Alere, San Diego, CA, USA), ITC Hemochron SIG+ POC (ITC Medical, Edison, NJ, USA), and the Roche Coaguchek XS POC Meter (Roche Diagnostics, Indianapolis, IN, USA). The aPTT assays were performed using the ITC Hemochron SIG+ POC. The key components and characteristics of the various PT and aPTT POC assay platforms are described in Table 1.

Subject screening

Eligible subjects were male and female volunteers, age 18–60 years, and in overall good health. All subjects provided written informed consent prior to inclusion in the study. Finger-stick blood samples were collected from healthy human subjects for the purpose of ascertaining the presence of a normal whole blood PT/INR using the Roche Coaguchek XS POC device. Subjects were excluded whose PT/INR did not fall within the manufacturer's reference range, who had participated in another clinical study in the previous 30 days, or who were currently taking agents known or suspected to alter platelet function or blood coagulation, such as aspirin, fish oil concentrates, anti-inflammatory medications, anti-histamines, anticoagulants, or antiplatelet drugs.

Sample collection

Eight healthy, eligible volunteers each underwent 4 venipunctures over a 15-min time period. At each venipuncture, approximately 3.5 ml of whole blood was collected. The initial 1.5 ml of blood was drawn into an empty syringe and discarded. The following 1.96 ml of blood was drawn into a second plastic syringe containing 40 µl of vehicle control or test agent. Therefore, after 4 venipunctures final telavancin concentrations of 0, 10, 20, or 100 µg/ml were achieved in four 2 ml samples of blood. Immediately following venipuncture, blood samples were gently mixed and analyzed within 30 s to minimize the risk of blood clotting. The concentrations were chosen to reflect clinically relevant plasma concentrations for the approved once-daily dose (10 mg/kg IV); 10 µg/ml telavancin corresponding to the expected trough level and 100 µg/ml reflecting the

Table 1 Point of care assays and instruments

Instrument	Detection method	PT				aPTT
		Thromboplastin source	Phospholipid used	ISI	INR reference range	Surface activator
Coaguchek XS	Electrochemical signal	Human (recombinant)	Yes	1.0	0.9–1.1	Not available on this device
Hemochron SIG+	Mechanical blood pump with optical detector	Rabbit (recombinant)	Yes	1.0	0.8–1.4	Kaolin
INRatio2	Electrical impedance	Human (recombinant)	No	1.0	0.7–1.2	Not available on this device
iSTAT	Electrochemical signal	Human (recombinant)	No	1.05	0.8–1.2	Not available on this device

INR international normalized ratio, *ISI* international sensitivity index

expected peak level [5]. At the time of the 4th venipuncture, an additional 2.7 ml of whole blood was collected into a 3.2 % sodium citrate tube and gently mixed. Citrated blood samples were mixed with 10, 20, or 100 μg/ml of telavancin (as above) or vehicle, before measuring aPTT using the Hemochron SIG+ instrument. Measurements of aPTT were made within 15 min of sample collection.

Statistical analysis

Statistical analysis of the PT and INR data was performed by two-way repeated measures analysis of variance including effects for telavancin concentration and POC devices (SigmaStat, version 3.5, Systat Software, San Jose, CA). Statistical analysis of the aPTT data was performed by one-way repeated measures analysis of variance including the effect of telavancin. For statistical analysis, p-values less than 0.05 were considered statistically significant and p-values less than 0.001 were considered highly statistically significant.

Results

The study population consisted of 4 male and 4 female subjects ranging in age from 22 to 62 years. Subjects self-identified as white (6), Asian (1), or African-American (1).

The effect of increasing concentrations of telavancin on PT is shown in Fig. 1. Statistically significant increases in PT were only observed at the 100 μg/ml concentration for three of the four POC assay platforms. In this small study, the Coaguchek XS assay was most affected at this concentration, with a mean increase in clotting time of 56 % over vehicle control (19.3 ± 2.1 vs. 12.4 ± 0.9 s; $p < 0.001$). Both the Hemochron SIG+ and the iSTAT assays were also significantly prolonged by 100 μg/ml telavancin, but to a lesser extent than the Coaguchek XS assay (PT prolonged approximately 15 % vs. control). The PT measured using the INRatio2 instrument was not significantly prolonged by telavancin over the concentration range tested.

The effect of increasing concentrations of telavancin on INR is shown in Fig. 2. The INR determined using Coaguchek XS was most affected by telavancin, increasing from a mean of 1.05 ± 0.08 with vehicle supplementation to 1.61 ± 0.17 at a concentration of 100 μg/ml ($p < 0.001$). The INR determined by Hemochron SIG+ was also notably impacted, increasing from 1.23 ± 0.07 with vehicle to 1.40 ± 0.17 at a concentration of 100 μg/ml ($p < 0.001$). Although statistically different, the INRs determined by INRatio2 and iSTAT were minimally increased compared with control. At lower concentrations of telavancin (10 and 20 μg/ml), INRs determined by iSTAT were also significantly increased compared with the vehicle, although the magnitude of increase was marginal.

The effect of telavancin supplementation on aPTTs measured using the Hemochron SIG+ is shown in Fig. 3. A concentration-dependent increase in clotting time was observed, with statistically significant increases in clotting time observed at concentrations greater than 20 μg/ml. At a concentration of 100 μg/ml, the aPTT was prolonged approximately 1.5-fold versus control. The effect of increasing concentrations of telavancin on PT/INR and aPTT are summarized in Table 2.

Discussion

A growing body of evidence demonstrates that telavancin prolongs in vitro clotting times [5, 7, 8]. In one study, Barriere et al. [5] reported that telavancin dose-dependently prolonged the PT and aPTT in vitro. The authors demonstrated that the degree of prolongation is dependent upon the composition of the reagents used. Barriere et al. [5] showed that for PT measurements, telavancin appeared to have a more pronounced effect when reagents containing recombinant tissue factor were used, compared with those containing tissue-derived thromboplastin. This difference in reagent source is important as a majority of POC coagulometers use recombinant thromboplastin for PT measurements. For the aPTT measurements in the same study,

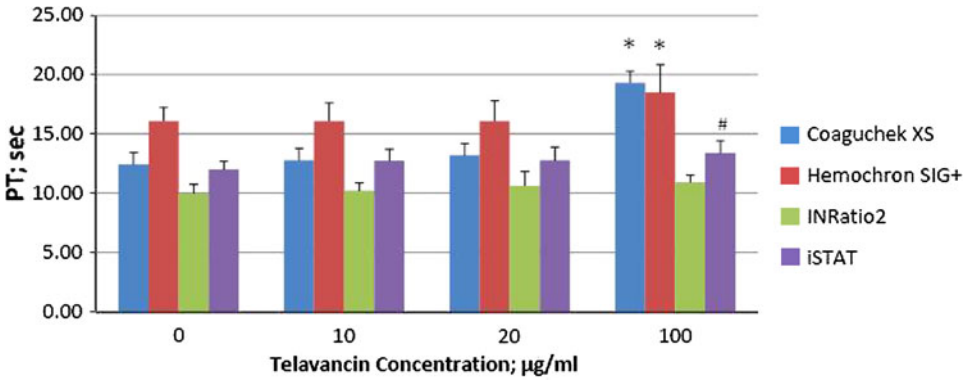

Fig. 1 Impact of telavancin on PT as measured by POC instruments. $*p < 0.001$ vs. 0 µg/ml; $^{\#}p = 0.003$ vs. 0 µg/ml. *POC* point-of-care, *PT* prothrombin time

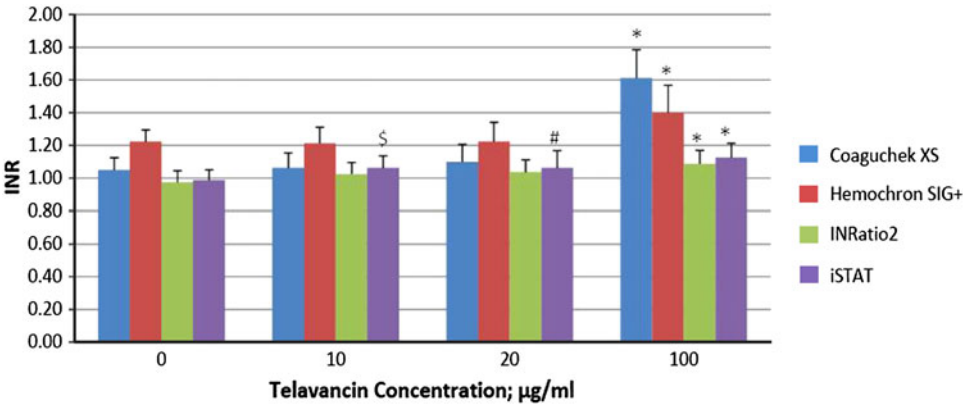

Fig. 2 Impact of telavancin on INR as measured by POC instruments. $*p < 0.001$ vs. 0 µg/ml; $^{\#}p = 0.021$ vs. 0 µg/ml; $^{\$}p = 0.008$ vs. 0 µg/ml. *POC* point-of-care, *INR* international normalized ratio

reagents containing a silica activator appeared more sensitive to the effects of telavancin than ellagic acid-based reagents. In the current study, the Hemochron SIG+ was the only device that offered aPTT testing and it uses a Kaolin surface activator. Gosselin et al. [7] reported similar reagent-based differences when examining PT and aPTT results. It has been hypothesized that telavancin prolongs in vitro clotting times by interfering with the phospholipid binding or with the assembly of coagulation factor complexes [5]. Differential interaction with phospholipids may explain why all reagents are not impacted to the same extent [7]. Similar results have been observed with the cyclic lipopeptide antibiotic daptomycin [9]. In that study, plasma supplemented with a range of daptomycin concentrations was analyzed with 30 different PT reagents containing rabbit brain, recombinant rabbit, recombinant human, or human placental thromboplastin on a Multi-Channel Discrete Analyzer (MDA) 180 automated coagulation analyzer. Daptomycin prolonged the PT measured with two of the 30 reagents tested. The aPTT and thrombin time did not appear to be affected by daptomycin supplementation.

In the current study, four POC instruments were used to measure PT/INR on blood samples supplemented in vitro with telavancin. As shown in Table 1, these instruments differed in detection method, manufacturer, and reagent composition. Of the POC instruments investigated in this study, three utilized recombinant human tissue factor and one utilized recombinant rabbit tissue factor which may contribute to assay variability between devices, but is outside the scope of this study. In half of the POC assay platform, phospholipid was incorporated in the activator. Low concentrations of telavancin, reflecting the expected trough level in patients treated once daily with telavancin, did not affect the PT/INR. At higher concentrations of telavancin, the assays that utilized phospholipid in the activator were most impacted. Mean INRs determined using the Coaguchek XS were increased by more than 50 %, whereas the INRs measured with the other devices were increased by 10–15 % over vehicle. Thus, in a clinical setting, The PT/INR monitoring may be impacted in telavancin-treated patients when monitored using the Coaguchek XS system. PT/INR monitoring using the iSTAT, Hemochron SIG+, and INRatio systems may also be impacted, but possibly to a lesser degree. These findings suggest that additional care may need to be employed when using the PT/INR POC instruments to monitor warfarin therapy in patients also receiving telavancin.

The aPTT assay is used to monitor heparin therapy. Prolongation of the aPTT of 1.5–2.5 × baseline is indicative of adequate heparinization. A concentration-dependent increase in aPTT was observed with telavancin with the

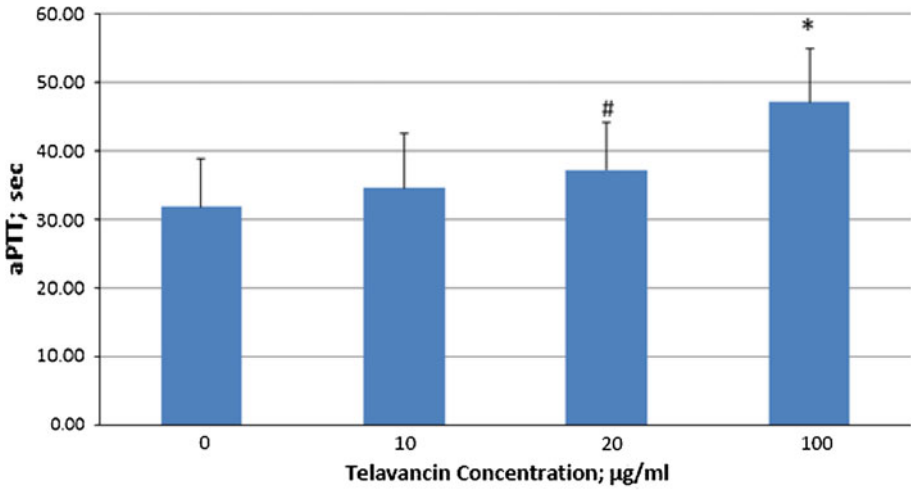

Fig. 3 Effect of telavancin supplementation on aPTT measured using the hemochron SIG+. $^{*}p < 0.001$ vs. 0 µg/ml; $^{\#}p = 0.018$ vs. 0 µg/ml. *aPTT* activated partial thromboplastin time

Table 2 The effect of increasing concentrations of telavancin on PT/INR and aPTT

Summary table of results									
Telavancin (µg/ml)	Coaguchek XS		Hemochron SIG+			INRatio2		iSTAT	
	PT (sec)	INR	PT (sec)	INR	aPTT (sec)	PT (sec)	INR	PT (sec)	INR
0	12.4 ± 0.9	1.1 ± 0.1	16.1 ± 1.2	1.2 ± 0.1	31.8 ± 7.0	10.0 ± 0.8	1.0 ± 0.1	11.9 ± 0.7	1.0 ± 0.1
10	12.8 ± 1.1	1.1 ± 0.1	16.1 ± 1.5	1.2 ± 0.1	34.6 ± 7.9	10.2 ± 0.7	1.1 ± 0.1	12.7 ± 1.0	1.1 ± 0.1
20	13.2 ± 1.0	1.1 ± 0.1	16.1 ± 1.7	1.2 ± 0.1	37.2 ± 6.8	10.6 ± 1.2	1.0 ± 0.1	12.8 ± 1.1	1.1 ± 0.1
100	19.3 ± 2.1	1.6 ± 0.2	18.5 ± 2.3	1.4 ± 0.2	47.1 ± 7.9	10.9 ± 0.6	1.0 ± 0.1	13.4 ± 1.0	1.1 ± 0.1

aPTT activated partial thromboplastin time, *INR* international normalized ratio, *PT* prothrombin time

Hemochron SIG+ assay. At peak telavancin levels, the aPTT was prolonged approximately 1.5-fold that of vehicle supplemented blood, suggesting a need for user awareness of the potential for artifactual prolongation of the clotting times.

The main limitation of this study lies in its small size and a larger trial would be needed confirm these preliminary findings. The use of citrated plasma for the aPTT POC test is a limitation as the standard protocol utilizes whole blood. Limitations also include the fact that no tissue-derived thromboplastin POC devices were tested within this study.

Overall, this study supports the current prescribing information recommendation that anticoagulant monitoring be conducted as close as possible prior to the next dose of telavancin. As this is the first study measuring the effects of telavancin on POC PT/INR and aPTT testing, future studies measuring POC testing with heparin or warfarin combined with telavancin will clarify their additive effects.

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Conflict of interest Michael P. Ero, Nathaniel R. Harvey, and Jack L. Harbert have no conflicts of interest to report. James W. Janc, Kay H. Chin, and Steven L. Barriere are employees of, and own equity securities of, Theravance, Inc.

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