Effect of Direct Thrombin Inhibitors, Bivalirudin, Lepirudin, and Argatroban, on Prothrombin Time and INR Values

Robert C. Gosselin, CLS,[1] William E. Dager, PharmD,[2] Jeffrey H. King, PharmD,[2] Kim Janatpour, MD,[1] Kathleen Mahackian, PharmD,[2] Edward C. Larkin, MD,[1] and John T. Owings, MD[3]

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Abstract

*Direct thrombin inhibitors (DTIs) represent a new class of promising anticoagulation agents. The DTIs frequently are used to provide initial anticoagulation, with long-term therapy requiring eventual transition to coumarins. Unfortunately, DTIs not only prolong the activated partial thromboplastin time but also can affect international normalized ratio (INR) values. We approximated the DTI effect on INRs by each drug to pooled plasma at concentrations between 0.1 and 1.2 µg/mL. We then concurrently tested these samples using 14 prothrombin time (PT) reagents. By using repeated measures analysis of variance, we found significant differences (*P < .05*) between the median INRs for lepirudin and argatroban for all PT reagents, between lepirudin and bivalirudin for all reagents except PT-Fibrinogen HS Plus (*P = .07*), and between bivalirudin and argatroban for all reagents except Thromborel S (*P = .05*). The DTI effect on INRs was dependent on drug, drug concentration, and reagent. Argatroban had the most effect on INRs, while lepirudin had the least effect. Reagents with a lower international sensitivity index were less affected by DTI; ThromboMax HS was the least sensitive PT reagent to any DTI.*

Direct thrombin inhibitors (DTIs) represent a new class of promising anticoagulation agents. The most common uses of parenteral DTIs are in the initial management of heparin-induced thrombocytopenia (HIT) and for anticoagulation in acute coronary syndromes.[1,2] The DTIs frequently are used to provide initial anticoagulation, with long-term therapy requiring eventual transition to coumarins. The presently available DTIs used in this setting are argatroban, bivalirudin, and lepirudin.

Because the DTIs have a fairly narrow therapeutic window, it is desirable to have a reliable means for measuring the intensity of anticoagulation. The activated partial thromboplastin time (aPTT) has served as the traditional means of monitoring the degree of anticoagulation for DTI regimens.[1,2] Within the target range, a rise in aPTT has a linear correlation to DTI plasma concentrations. With the exception of percutaneous coronary intervention (PCI), the target ranges for thromboprophylaxis or treatment of thromboembolism are aPTT ratios from 1.5 to 2.5 or 3.0, depending on the individual agent. Data for these target aPTT ratios were provided mostly from trials using DTIs in the management of HIT. Unfortunately, DTIs not only prolong the aPTT, but also can have some crossover effect on international normalized ratio (INR) values,[3-5] which can create a challenge when making the transition to warfarin therapy during concurrent DTI administration. The presently available data on the INR elevation are limited to a few reagents used to measure the prothrombin time (PT) and the concurrently reported INR. In the present study, we added DTIs to pooled plasma at various concentrations to estimate the relative anticoagulation effect of these agents on 14 PT reagents available in the United States.

Materials and Methods

Bivalirudin (The Medicines Co, Parsippany, NJ), lepirudin (Berlex Laboratories, Montville, NJ), and argatroban (Texas Biotech, Houston, TX) were prepared according to the manufacturers' instructions. By using class A volumetric pipettes, each drug was added to a pooled normal plasma preparation (from at least 20 normal donors) to achieve concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 µg/mL. An aliquot of the pooled normal plasma and each drug–pooled normal plasma preparation was frozen at –70°C before testing. For each sample, PT measurements were performed using the reagents listed in **Table 1**.

Testing of pooled plasma (baseline) and DTI samples was performed using the MLA 900C (Medical Laboratory Automation, Pleasantville, NY) or the CA-1500 (Dade Behring, Newark, NJ). PT reagents were prepared fresh on the day of use, and all DTI sample preparations were tested on each reagent system concomitantly to avoid day-to-day bias. Within-run precision studies for the PT and aPTT methods also were performed for each analyzer, by concurrently analyzing 10 normal and abnormal samples using a sensitive PT and aPTT reagent.

To eliminate potential reagent bias seen with reagents used in PT testing, calculation of PT ratios was done by using the baseline result for each reagent. However, PT ratios do not accurately reflect the relative sensitivity of each reagent, especially because INR reporting is the standard of practice. Therefore, relative INR calculations were made by using the obtained ratios and manufacturer-provided international sensitivity index (ISI) values. The ISI values and reference means used in this study are listed in Table 1. Each sample was tested in duplicate, and the mean of the replicates was used for statistical analysis.

To determine the anticoagulation potential of each drug at these concentrations, aPTT testing using Actin FSL (Dade Behring) was performed concurrently on the CA-1500, and ratios were calculated for each drug concentration. Ratios were calculated from baseline (pooled plasma) and resultant aPTTs from DTI-plasma samples. The typical (non-PCI) recommended therapeutic target aPTT ratios for lepirudin and argatroban anticoagulation are between 1.5 and 2.5 and 1.5 and 3.0 (but not to exceed 100 seconds), respectively. Bivalirudin is approved in the United States only for use in patients undergoing PCI, but lower dose concentrations with similar aPTT ratios to other DTIs are being explored for the prevention and treatment of thromboembolism and HIT.[6]

Repeated measures analysis of variance was used to determine whether differences existed between the pooled data for different reagents and different DTIs and whether differences existed within each reagent and different DTI. The Pearson correlation was performed to determine the correlation between PT values and increasing drug concentrations. A P value of less than .05 was considered statistically significant.

Results

The maximum coefficient of variation (CV) for the within-run precision of PT measurements using the MLA 900C was 2.2%, while the maximum CV for the CA-1500 PT measurement was 1.5%. The maximum CV for aPTT testing on the CA-1500 was 1.0%.

By using the aPTT ratio as the benchmark measurement for DTI anticoagulation, the therapeutic DTI concentration of more than 0.2 µg/mL was consistent with the therapeutic range **Table 2**, with mean ± SD aPTT ratios ranging from

Table 1
Manufacturers or Distributors of Reagents Used in the Study*

Reagent	Manufacturer or Distributor	ISI	Mean PT (s)
PT-Fibrinogen Recombinant	Beckman Coulter, Miami, FL	1.11	13.1
PT-Fibrinogen	Beckman Coulter	1.87	13.1
PT-Fibrinogen HS	Beckman Coulter	1.42	13.3
PT-Fibrinogen HS Plus	Beckman Coulter	1.15	14.6
RecombiPlasTin	Beckman Coulter	0.97	12.0
Simplastin HTF	bioMérieux, Marcy l'Etoile, France	1.27	10.8
Innovin	Dade Behring, Newark, NJ	1.03	10.5
Thromboplastin C Plus	Dade Behring	1.86	11.0
Thromborel S	Dade Behring	0.92	10.7
PT-One	PharmaNetics, Morrisville, NC	1.05	11.2
Neoplastine	Stago International, Parsippany, NJ	1.75	11.9
Neoplastine Plus	Stago International	1.33	12.2
ThromboMax	Trinity Biotech, Bray, Ireland	1.54	10.5
ThromboMax HS	Trinity Biotech	1.07	14.1

PT, prothrombin time.
* The international sensitivity index (ISI) value used for international normalized ratio (INR) determination was specific for instrumentation or selected by method. The reference mean used for INR calculation was the mean for each respective reagent on the pooled plasma sample used for direct thrombin inhibitor testing.

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Table 2
Pooled Prothrombin Time Ratio Data (95% Confidence Intervals) for All Reagents Used in the Study*

Direct Thrombin Inhibitor Concentration (µg/mL)	Lepirudin	Bivalirudin	Argatroban
0.1	1.03† (1.02-1.04)	1.06 (1.04-1.08)	1.09 (1.07-1.11)
0.2	1.06† (1.04-1.08)	1.09 (1.07-1.11)	1.15 (1.12-1.18)
0.4	1.09† (1.07-1.11)	1.15† (1.12-1.18)	1.33 (1.26-1.40)
0.6	1.13† (1.10-1.16)	1.23† (1.19-1.28)	1.46 (1.36-1.56)
0.8	1.16† (1.12-1.20)	1.27† (1.22-1.32)	1.66 (1.54-1.78)
1.0	1.19† (1.15-1.23)	1.34† (1.27-1.41)	1.84 (1.70-1.98)
1.2	1.20† (1.16-1.24)	1.39† (1.31-1.47)	2.05 (1.88-2.22)

* Ratios were calculated using the prothrombin time values obtained from direct thrombin inhibitor plasma and the baseline prothrombin time values noted in Table 1.
† $P < .05$; repeated measures analysis of variance on ranks between lepirudin and bivalirudin ratios with argatroban ratios.

1.2 ± 0.1 (for the 0.1 µg/mL DTI concentration) to 2.2 ± 0.1 (for the 1.2 µg/mL DTI concentration). As expected, with increasing DTI concentrations, there was a linear increase effect on the PT values. By using the pooled ratio data from all PT reagents, we found significant differences for PT ratios between lepirudin and argatroban at all concentrations and between bivalirudin and argatroban at concentrations of more than 0.2 µg/mL (Table 2).

To compare the relative effect of each DTI on each PT reagent, a median INR was calculated from the INR values obtained from the DTI samples **Table 3**. There were significant differences ($P < .05$) between the median INR for lepirudin and argatroban for all PT reagents. There also were significant differences ($P < .05$) between the median INR for lepirudin and bivalirudin for all reagents except PT-Fibrinogen HS Plus ($P = .07$). Significant differences ($P < .05$) in the median INR between bivalirudin and argatroban for all reagents except Thromborel S also were observed ($P = .05$). All PTs correlated significantly ($R > 0.90$) with increasing drug levels ($P < .005$). The ranges of PT ratio results for the DTI plasma samples for each set of reagent systems are plotted in **Figure 1**. ThromboMax HS was the least sensitive PT reagent to any DTI. Innovin and RecombiPlasTin were relatively insensitive to the presence of either lepirudin or bivalirudin.

Discussion

DTIs such as argatroban, bivalirudin, and lepirudin are being used increasingly in place of heparin to provide initial, rapid anticoagulation. Unlike heparin (or low-molecular-weight heparin), which requires a mediator (antithrombin) to potentiate anticoagulation, DTIs can inhibit free and bound thrombin directly. Lepirudin is a recombinant hirudin derived from yeast cells and forms an equimolar complex

Table 3
Median INR (95% Confidence Interval) for Each Reagent Calculated From the INRs Obtained From the Range of Drug Concentrations*

Reagent	Lepirudin	Bivalirudin	Argatroban
PT-Fibrinogen Recombinant	1.24†‡ (1.11-1.37)	1.50† (1.21-1.79)	2.12 (1.44-2.80)
PT-Fibrinogen	1.32†‡ (1.18-1.46)	1.53† (1.26-1.80)	1.85 (1.33-2.37)
PT-Fibrinogen HS	1.27†‡ (1.14-1.40)	1.39† (1.17-1.61)	1.89 (1.33-2.45)
PT-Fibrinogen HS Plus	1.22† (1.14-1.30)	1.27† (1.13-1.41)	1.59 (1.26-1.92)
RecombiPlasTin	1.08†‡ (1.03-1.13)	1.15† (1.07-1.23)	1.23 (1.00-1.48)
Simplastin HTF	1.17†‡ (1.09-1.25)	1.36† (1.17-1.55)	1.68 (1.23-2.13)
Innovin	1.03†‡ (1.00-1.06)	1.10† (1.03-1.17)	1.34 (1.00-1.79)
Thromboplastin C Plus	1.17†‡ (1.09-1.25)	1.30† (1.15-1.46)	1.76 (1.00-2.67)
Thromborel S	1.17†‡ (1.11-1.23)	1.23 (1.12-1.34)	1.37 (1.06-1.68)
PT-One	1.12†‡ (1.06-1.19)	1.25† (1.14-1.36)	1.59 (1.26-1.92)
Neoplastine	1.20†‡ (1.09-1.32)	1.42† (1.22-1.62)	1.88 (1.03-2.73)
Neoplastine Plus	1.16†‡ (1.07-1.25)	1.32† (1.17-1.47)	1.76 (1.02-2.50)
ThromboMax	1.13†‡ (1.07-1.19)	1.26† (1.13-1.39)	1.53 (1.00-2.15)
ThromboMax HS	1.12†‡ (1.06-1.18)	1.28† (1.15-1.41)	1.32 (1.14-1.50)

INR, international normalized ratio.
* For proprietary information, see Table 1.
† There were significant differences ($P < .05$) between lepirudin and argatroban. All bivalirudin INRs were significantly different ($P < .05$) from argatroban INRs except with Thromborel S.
‡ All lepirudin INRs were significantly different ($P < .05$) from bivalirudin INRs except with PT-Fibrinogen HS Plus.

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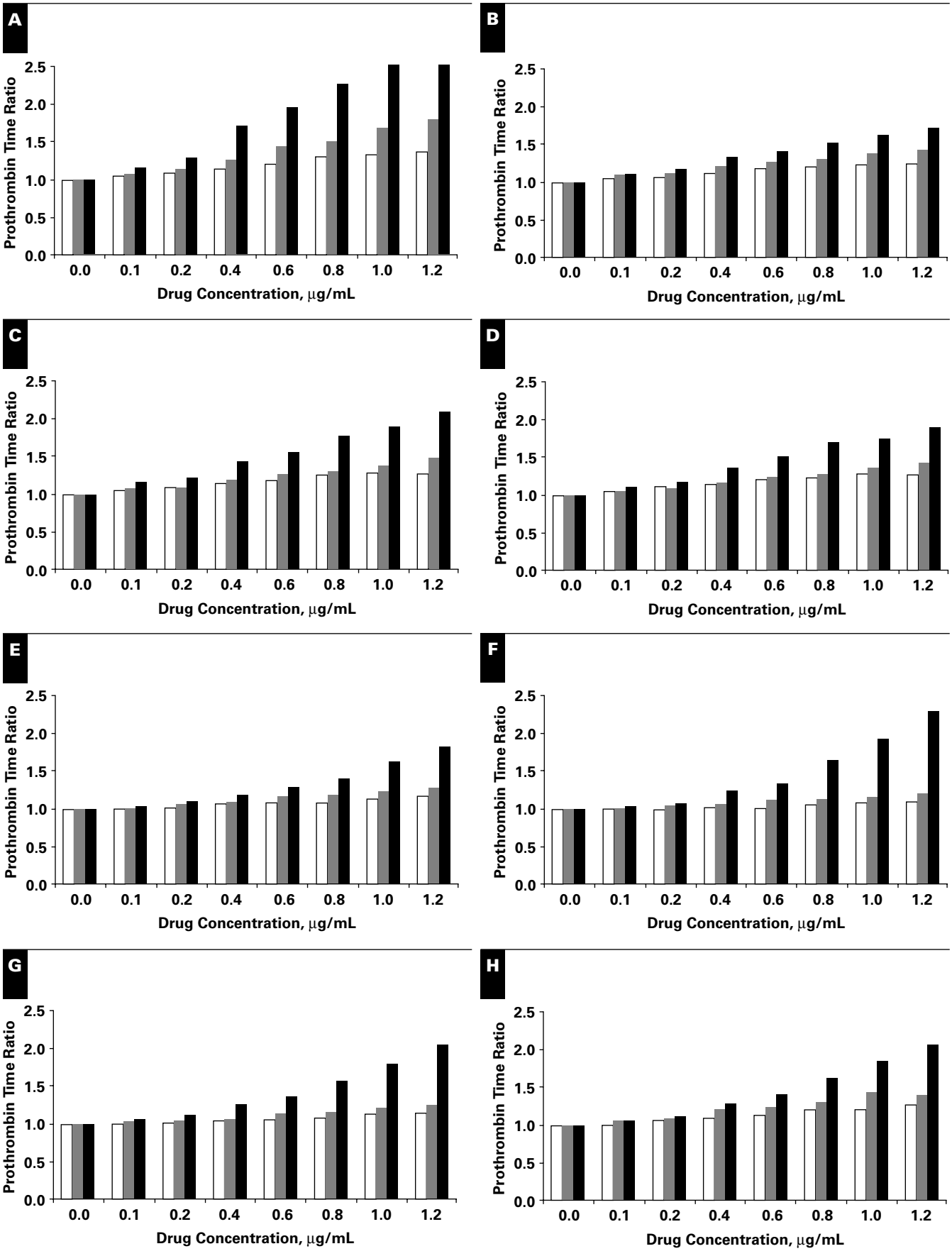

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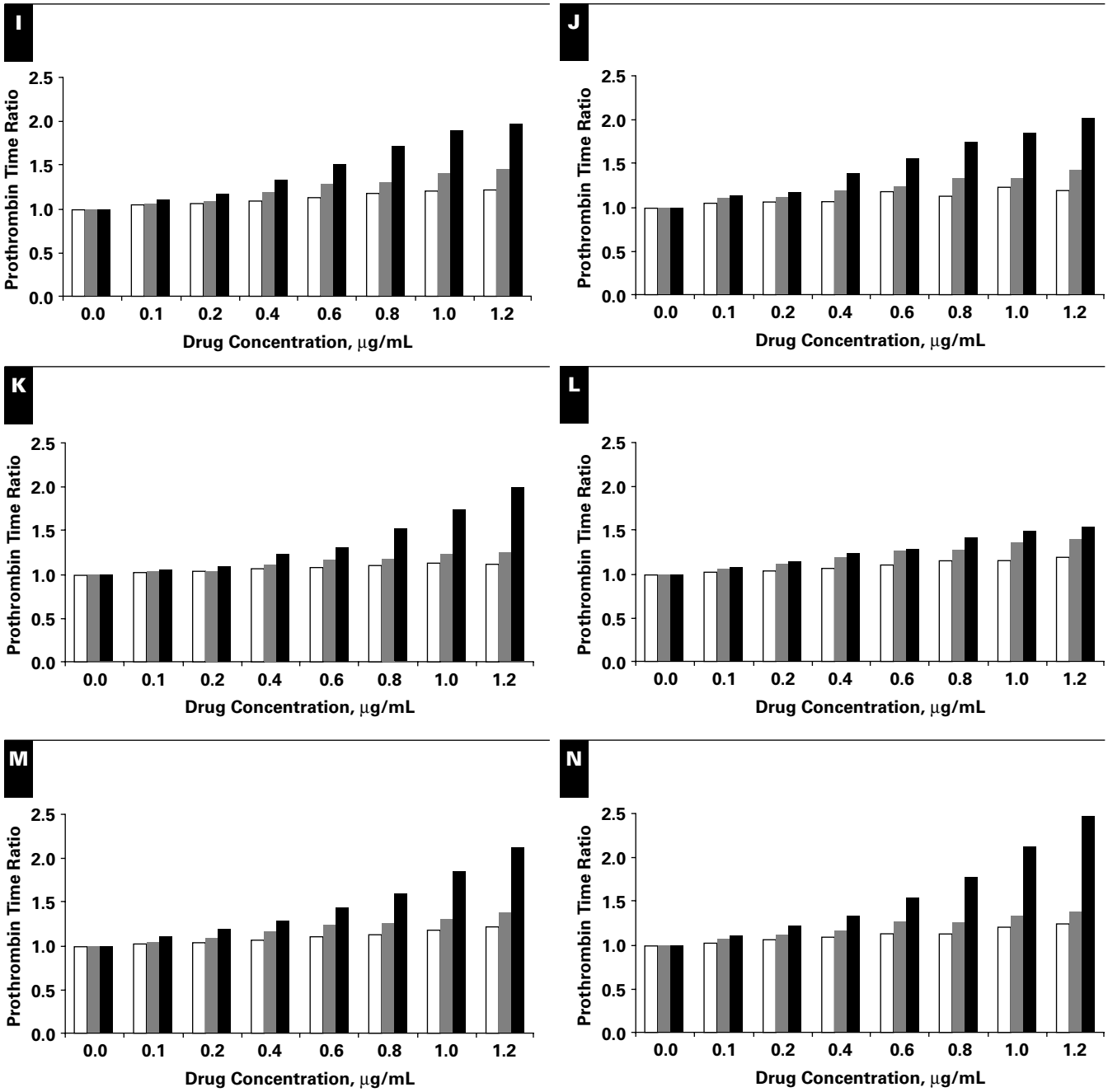

Figure 1 Linear representation of the effect of lepirudin (white bars), bivalirudin (gray bars), and argatroban (black bars) on prothrombin time reagents. **A**, PT-Fibrinogen Recombinant. **B**, PT-Fibrinogen. **C**, PT-Fibrinogen HS. **D**, PT-Fibrinogen HS Plus. **E**, RecombiPlasTin. **F**, Innovin. **G**, Thromboplastin C Plus. **H**, Thromborel S. **I**, Simplastin HTF. **J**, PT-One. **K**, ThromboMax. **L**, ThromboMax HS. **M**, Neoplastine. **N**, Neoplastine Plus. For proprietary information, see Table 1

with thrombin to inhibit thrombin's activity. Bivalirudin is a synthetic analog of hirudin, with a shorter period of binding to thrombin. Both agents bind not only to the active (catalytic) site but also to the fibrinogen (anion-binding exosite) site. Argatroban is a synthetic inhibitor of thrombin derived from L-arginine, which has a relatively short period of binding only to thrombin's active site.

In the present study, we sought to determine the effect of DTIs at various concentrations on the PT and subsequent INR calculations. There are a few reports of the effects of argatroban on INR results,[3-5,7] but there are limited data on the effect of lepirudin and bivalirudin on INR values.[5] To our knowledge, there have been no studies looking at lepirudin, bivalirudin, and argatroban concurrently to determine the

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relative effect of DTIs on INR values. Reports looking at the effects of argatroban on the INR indicate a linear relationship with patients receiving increasing argatroban concentrations or concomitant argatroban and warfarin therapy.[3,4]

In lieu of actual clotting times, evaluating PT ratio data can identify the relative differences between reagent systems. We noted significant differences between all 3 DTIs and the PT ratios (Table 2). All PT ratios increased in a dose-dependent manner for all DTI samples. The relative changes in PT ratios were less pronounced with reagents with lower ISIs, Innovin and RecombiPlasTin, but other reagents with a lower ISI, such as Recombinant PT-Fibrinogen and PT-Fibrinogen HS Plus, demonstrated significant bias in the INR for argatroban samples.

The increasing dose-dependent effect of argatroban on the INR also is dependent on reagent sensitivity.[3,4] Hursting and colleagues[4] described the relationship between PT reagent sensitivity, as expressed by ISI values, and the degree of the prolongation of the INR. Their conclusions indicated that using reagents with lower ISIs diminished the effect of argatroban on the INR.

In our study, we observed a dose-dependent effect on the INR with lepirudin, argatroban, and bivalirudin. At the highest drug concentration (1.2 μg/mL), lepirudin affected the PT values the least, with INR values ranging from a low of 1.07 to a high of 1.42 for Innovin and PT-Fibrinogen Recombinant, respectively (Figure 1). At the highest drug concentration for argatroban, ThromboMax HS and RecombiPlasTin were least affected, with INRs of 1.62 and 1.78, respectively. At the highest drug concentration, there were statistical differences between the effect of lepirudin and bivalirudin on the INR for the Innovin and RecombiPlasTin reagents. However, it is unclear whether these noted statistical differences or the INR recorded at the highest concentration with these reagents (INR approximately 1.2) is clinically significant.

As noted with previous studies,[3,4] there was a greater effect on the INR calculations for DTI samples when using reagents with higher ISI values. All PT reagents used in the present study demonstrated a more significant dose-dependent response with argatroban than with lepirudin or bivalirudin.

To our knowledge, there have been no studies that have addressed the use of DTIs in patients with elevated aPTTs in the absence of heparin or other drug therapy. The most common cause for the prolongation of the aPTT in these patients is the presence of a lupus anticoagulant. There is a correlation between the degree of aPTT prolongation and circulating levels of lupus anticoagulants. Because lupus anticoagulants have been associated with thrombotic risk, development of HIT in these patients who are treated with heparin is a distinct possibility. To our knowledge, there are no published data on the concomitant effect of lupus anticoagulants and DTIs on aPTT test results. In the absence of a phospholipid antibody as the cause for the prolongation of the PT, it seems plausible that the treatment for patients with lupus anticoagulants who receive argatroban might be managed more easily with PT measurements, because there is a dose-dependent linear relationship between PT values and argatroban levels.

There are 2 potential weaknesses to our study. First, we used pooled normal plasma and added known amounts of the DTIs to achieve the desired concentrations. In other anticoagulation studies, it is generally accepted that spiking pooled plasma with an anticoagulant (eg, heparin) is not an effective means for determining the dose response of the drug. This is most likely due to the variation in clotting factor levels that might affect a given laboratory test result such as the PT or aPTT and to responses to the anticoagulation effect of the drug itself between individuals. The present study was not intended to provide dose responses but to determine the relative effect that DTIs have on PT and INR values. This information is useful to clinicians to weigh the possibility of biased INR results depending on the thromboplastin used during DTI therapy. However, as an in vitro study, these data provide an approximation of the DTI effect on PT and INR values. Confirmation of these biases is necessary by analyzing samples obtained from patients receiving these drugs.

The second weakness of this study is that we reported INRs that have not been validated on the testing instrument. To correctly ascertain an INR, the laboratory must establish a normal reference mean on samples from at least 40 ostensibly healthy people. In addition, the ISI should be instrument specific to achieve greater accuracy. In the present study, we used the pooled normal plasma PT results as the reference mean for our INR calculations. In addition, we selected from the manufacturer's provided ISI table the most appropriate value for the method used. In the present study, all INR values were calculated by using the respective reference means and selected ISI values. While this may not be the most acceptable means of assessing the INR, any error in INR determination would have been systematic for all values within that reagent system. It is highly likely that a laboratory will use a single PT reagent, yet test samples from patients receiving lepirudin, argatroban, or bivalirudin. Therefore, this study answers the relative INR effect for patients receiving these DTIs for a given PT reagent. The accuracy of the INRs in this study is more questionable when comparing the effect of DTIs on different PT reagents, because the potential INR bias that might be systematic within a reagent might not be an equivalent error for other reagents. The use of more than one PT reagent in a clinical laboratory would not be a common practice, and the information of

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differing effects on INR reporting for different reagent systems would be of greater interest to clinicians who might serve patients at more than one institution.

Conclusions

DTIs have a dose-dependent effect on PT and INR values. PT and INR values seem to be affected most by argatroban at therapeutic concentrations, which might complicate the transition to warfarin therapy. Lepirudin had the least overall effect on PT and INR values. Reagents with a lower ISI, especially ThromboMax HS, Innovin, and RecombiPlasTin, were least affected by the presence of DTIs. This study is an in vitro approximation of the dose-dependent effect of DTIs on PT and INR values. Confirmation of the observed DTI effect on PT and INR values might require additional evaluation using samples from patients receiving these drugs.

From the Departments of [1]Pathology, [2]Pharmacy, and [3]Surgery, University of California, Davis Medical Center, Sacramento, CA.

Address reprint requests to Dr Owings: UCDMC Trauma Division, Room 4209, 2315 Stockton Blvd, Sacramento, CA 95817.

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