Presence of Direct Thrombin Inhibitors Can Affect the Results and Interpretation of Lupus Anticoagulant Testing

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Key Words: Dilute Russell viper venom test; Lupus anticoagulant; Antiphospholipid antibody syndrome; Direct thrombin inhibitor; Platelet neutralization procedure; Heparin; Coumadin; Warfarin

Abstract

In vitro experiments were conducted to determine whether the direct thrombin inhibitors argatroban and lepirudin can interfere with the results of lupus anticoagulant (LA) testing. Concentration-response curves were generated to calculate the concentration of anticoagulant that prolongs the activated partial thromboplastin time (aPTT) to 75 seconds (2.5 times the baseline average). Corresponding concentrations of anticoagulant were added to plasma samples before running dilute Russell viper venom time (DRVVT) and LA-sensitive aPTT (PTT-LA) tests. Because the DRVVT test system contains an antiheparin agent, DRVVT results were not prolonged in the presence of heparin. With argatroban added to normal plasma samples, neither the DRVVT percent correction of ratio nor the DRVVT test/confirm ratio was elevated, but when added to LA-positive plasma, some false-negative results were observed. Lepirudin increased the DRVVT percent correction of ratio and the DRVVT test/confirm ratio into a range that could lead to false-positive identifications of LAs. In sharp contrast to the DRVVT test system, distinction between LA-positive and LA-negative plasma samples was maintained and possibly even enhanced in the platelet neutralization procedure correction phase of the PTT-LA test system.

The antiphospholipid antibody syndrome is a clinical diagnosis characterized by vascular thrombosis or pregnancy morbidity observed in combination with laboratory evidence of antiphospholipid antibodies or lupus anticoagulants (LAs). The term antiphospholipid antibody is a misnomer because the antibodies likely are directed toward phospholipid-binding proteins such as β2-glycoprotein I or prothrombin in combination with phospholipids such as cardiolipin, phosphatidylserine, or phosphatidylethanolamine.

Antiphospholipid antibodies typically are detected by using solid-phase assays. Related antibodies capable of prolonging clotting times in phospholipid-dependent coagulation assays are termed lupus anticoagulants. Essential criteria for the diagnosis of an LA are as follows: (1) a prolonged phospholipid-dependent clotting test result, (2) failure of mixing studies to correct the abnormality, (3) decrease of the level of inhibition in the presence of high-concentration phospholipid, and (4) an absence of inhibitors to specific coagulation factors that would account for the prolongation. It has been recommended that at least 2 different coagulation-based assays be used to test a patient's plasma sample. Because LAs determined by clotting assays might constitute a stronger risk factor for thrombosis than antibodies detected by solid-phase assays, clotting assays remain central in the testing for antiphospholipid syndrome antibodies.

Patients admitted to the hospital with thrombosis often require immediate and prolonged anticoagulation, regardless of whether the thrombotic event was due to the antiphospholipid antibody syndrome. This presents a diagnostic dilemma: once anticoagulant therapy has been initiated, is it still possible to diagnose an LA? Heparin, for example, has been shown to prolong the dilute Russell viper venom time...
(DRVVT) result,15 as well as partial thromboplastin time (PTT)-based LA test results,16 including those incorporating the platelet neutralization procedure (PNP).17 Therefore, antiheparin agents are added to some commercial DRVVT kits.18 The oral anticoagulant warfarin has been reported to cause weakly false-positive DRVVT results,19 although it also has been reported that LA testing may be feasible in the presence of warfarin.20

Direct thrombin inhibitors, however, are being used increasingly as alternative anticoagulants.21 These drugs may present new challenges for the monitoring of appropriate anticoagulation,22 and early studies have demonstrated that direct thrombin inhibitors might interfere with a number of coagulation-based assays, including testing for LAs using the DRVVT 23,24 or PTT-based assays.25

The present in vitro experiments were conducted to determine whether the commercially available direct thrombin inhibitors argatroban (a synthetic derivative of arginine) and lepirudin (a recombinant form of hirudin) interfere with accurate LA testing in samples of plasma from healthy donors or LA-positive patients. Two LA assays, a modified DRVVT and an LA-sensitive PTT (PTT-LA) that employs the PNP,17 were studied.

Materials and Methods

Procedures

Investigations were conducted in accordance with local institutional review board guidelines. Normal plasma was obtained with informed consent from blood donors with no personal or family history of abnormal bleeding or clotting and no recent use of drugs such as aspirin, ibuprofen, or other anticoagulants. Venous blood was collected into 5-mL specimen tubes (BD Vacutainers, Becton Dickinson, Mountain View, CA) prefilled with 0.5 mL of 3.2% buffered sodium citrate (0.105 mol/L). These blood samples subsequently were centrifuged at 4440 g for 4 minutes to prepare platelet-poor plasma. This centrifugation consistently resulted in fewer than 10,000 platelets per microliter, consistent with National Committee for Clinical Laboratory Standards guidelines.26 Aliquots of plasma were frozen in Cryotubes (Corning, Corning, NY) at −80°C.

For the experiments, plasma was thawed for 5 minutes at 37°C in a water bath and briefly agitated on a vortex mixer. Activated PTTs (aPTTs) were performed using standard aPTT kit reagents (No. 12203, Diagnostica Stago, Parsippany, NJ) on an STA Compact Coagulation Analyzer (Diagnostica Stago) according to the manufacturer’s instructions. Quality control measurements were in range according to standard clinical laboratory protocol before testing. DRVVVTs were performed using LA Screen and LA Confirm kits (Gradiopore, Hawthorne, NY) according to the manufacturer’s instructions.

The lupus-positive control sample was from Precision Biologic (Dartmouth, Canada). The PTT-LA test (reagent No. 0599, Diagnostica Stago) was performed with the addition of platelet lysate or an equivalent volume of 0.05 mol/L of tris(hydroxymethyl)aminomethane-buffered saline, prepared using Trizma base and sodium chloride (Sigma, St Louis, MO), with the pH set to 7.5.

Identification of Lupus-Positive Samples

Deidentified, frozen (−80°C) samples of adult plasma previously identified as positive for LAs in the absence of oral or parenteral anticoagulant therapy were used in the study. Inclusion criteria included an initial positive DRVVT test/confirm ratio. Each of these samples also had a borderline or frankly positive dilute tissue thromboplastin inhibition test result,27 and 6 of 9 samples had a positive anticardiolipin antibody or an anti–β2-glycoprotein antibody as detected by enzyme-linked immunosorbent assay. After thawing, all samples retained their original positive states with respect to the DRVVT test/confirm ratio. It is interesting that when using the new percent correction of ratio calculation (see the “Analysis” section), 2 of these samples would be reclassified as negative. These 2 samples were included in the present analysis to examine the effects of direct thrombin inhibitors on borderline results.

Preparation of Drug Solutions

Lepirudin was supplied as a freeze-dried powder containing 50 mg of lepirudin, 40 mg of mannitol, and sodium hydroxide to adjust the pH of the reconstituted material to approximately 7.0. Lepirudin was dissolved in 0.9% sodium chloride (Braun, Irvine, CA) and was stored in aliquots at −80°C. Argatroban was supplied as a solution containing 100 mg of argatroban, 750 mg of D-sorbitol, and 1,000 mg of dehydrated alcohol. Argatroban was stored at room temperature. Porcine heparin was supplied as 1,000 USP units of heparin sodium, 9 mg of sodium chloride, 0.15% methylparaben, and 0.015% propylparaben dissolved in isotonic saline (American Pharmaceutical Partners, Schaumburg, IL) and was stored at 4°C.

Daily working drug solutions of varying concentrations were prepared in 0.9% sodium chloride, and stock solutions were chosen such that 5 µL of drug solution always was added to 1 mL or 1.5 mL of patient plasma. This procedure allowed precise calculation of the final drug concentration in plasma with minimal dilution.

Reagents

We obtained 0.9% sodium chloride irrigation USP from Braun. STA aPTT, Ca²⁺Cl₂, and PTT-LA reagents were obtained from Diagnostica Stago. Heparin sodium, lepirudin, and argatroban were obtained from the hospital pharmacy. Platelet lysate, a PNP reagent, was obtained from Precision Biologic and was prepared according to previously described methods.17
Genzen and Miller / Direct Thrombin Inhibitors and LA Testing

Analysis

The relationship between aPTT and drug concentration was determined using nonlinear regression analysis in SigmaPlot (SPSS, Chicago, IL). DRVVT data were calculated using multiple methods. The DRVVT ratio was calculated as follows:

$$\text{DRVVT Ratio} = \frac{\text{Test DRVVT}}{\text{Control DRVVT}}$$

LAs (as well as some exogenous anticoagulants) cause an increased DRVVT ratio. This ratio is different from the commonly reported test/confirm ratio, which is calculated as follows:

$$\text{Test/Confirm Ratio} = \frac{\text{Test DRVVT}}{\text{Test DRVVT With } \uparrow \text{ Phospholipid}}$$

The test/confirm ratio is a measurement of correction in the presence of high-concentration phospholipid. LAs increase the test/confirm ratio, although this ratio does not control for variations in reagent or operator performance. The percent correction of ratio, in contrast, not only expresses the amount of correction observed in the DRVVT in the presence of high-concentration phospholipid but also normalizes these results for potential variations in reagents and operator performance.

Equation II. It is calculated as shown

$$\text{Equation II.}$$

The DRVVT reference ranges were derived from 20 donors and were calculated as the mean ± 2 SD. Reference ranges were as follows: DRVVT ratio, 0.75 to 1.05; percent correction of ratio, −10% to +15%; and test/confirm ratio, 0.94 to 1.17. An LA results in an increased DRVVT ratio, percent correction of ratio, and test/confirm ratio.

The PTT-LA reactions use a lupus-sensitive PTT reagent and a platelet lysate to test for a prolonged phospholipid-dependent clotting assay result. The reference range for the baseline PTT-LA, 30.3 to 46.4 seconds, was derived from a population of 30 donors (mean ± 2 SD). The PTT-LA correction is defined as follows:

$$\text{PTT-LA Correction} = \text{PTT-LA}_{\text{saline}} - \text{PTT-LA}_{\text{lysate}}$$

The reference interval for the PTT-LA correction derived from these same donors was −2.5 to +1.0 seconds, ie, with most normal plasma samples, incubation with the platelet lysate slightly prolonged the PTT-LA, resulting in a negative value for this calculation. As a test of reproducibility for this procedure, a known LA-positive plasma sample with an initial PTT-LA of 53.5 seconds and a PTT-LA correction of +3.4 seconds was frozen and then thawed, and 10 replicate assays were performed. The mean of these PTT-LAs was 53.9 seconds (SD, 1.3 seconds). The mean of the PTT-LA corrections was +3.9 seconds (SD, 0.5 second). Of particular note, the observed variation in the replicate PTT-LA correction values did not exceed 1.1 seconds, a value of insufficient magnitude to change the classification between negative and positive for any of the control or LA-positive plasma samples used in the present study.

Raw data were imported into Excel (Microsoft, Redmond, WA) and SigmaPlot for calculations, statistical analysis, and display. Data are given as mean ± SD unless otherwise indicated.

Results

Initial experiments were directed toward establishing the effects of direct thrombin inhibitors on the aPTT (reference range, 23.1−36.5 seconds) since the aPTT commonly is used to monitor these agents in patients without LAs. An aPTT prolongation of 2.5 times the baseline equals or exceeds the usual anticoagulant target intensity for each of the thrombin inhibitors studied. The mean baseline aPTT was determined to be 30.1 ± 1.4 seconds (n = 9), and 2.5 times the baseline was, accordingly, 75 seconds. Increasing concentrations of heparin (Figure 1A), argatroban (Figure 1B), and lepirudin (Figure 1C) were added to samples of normal plasma before running aPTTs on the coagulation analyzer (n = 5 or 6 per concentration). Raw data points were fit using nonlinear regression. The calculated concentrations required to prolong the aPTT to 75 seconds were as follows: heparin, 0.4 U/mL; argatroban, 1.24 µg/mL; and lepirudin, 1.67 µg/mL (Figure 1, dotted lines). As further confirmation, these calculated concentrations of drugs were added back to plasma and yielded aPTTs of 78.7 ± 21.2 seconds for heparin, 76.3 ± 9.3 seconds for argatroban, and 71.3 ± 3.9 seconds for lepirudin. Therefore, these concentrations were used in subsequent experiments, except as otherwise noted.

To determine whether anticoagulants interfere with the DRVVT, anticoagulants were added to normal donor plasma before running these reactions on the coagulation analyzer. The DRVVT ratio is simply a calculation capable of demonstrating a prolonged DRVVT result compared with a control (see the “Materials and Methods” section). The Gradipore DRVVT kit contains an antiheparin agent, which effectively prevented prolongation in the presence of 0.4 U/mL of heparin (DRVVT ratio before, 0.96 ± 0.1; with heparin, 0.95 ± 0.08; n = 6; P = .14; data not shown). As expected, the test/confirm ratio (before, 1.0 ± 0.1; with heparin, 1.0 ± 0.1; n = 6; P = .81) (Figure 2A) and the percent correction of ratio (before, 1.9% ± 4.7%; with heparin, 1.8% ± 3.9%; n = 6; P = .87) (Figure 2B) did not change.
Figure 1 Prolongation of activated partial thromboplastin time (aPTT) with the addition of anticoagulants to normal plasma. Dose-response curves demonstrating the prolongation of aPTTs in the presence of (A) heparin, (B) argatroban, and (C) lepirudin. Data points were fit using nonlinear regression, which yielded the following equations: heparin, \( y = 29.33e^{2.333x} \), \( r^2 = 0.99 \); argatroban, \( y = 40.63x^{1/2} + 29.73 \), \( r^2 = 0.98 \); lepirudin, \( y = 32.37x^{1/2} + 33.28 \), \( r^2 = 0.99 \). Dotted lines indicate the calculated concentration of anticoagulant predicted to cause an increase in aPTT 2.5 times the baseline average of 30.1 ± 1.4 seconds (n = 9) and were as follows: heparin, 0.4 U/mL; argatroban, 1.24 µg/mL; and lepirudin, 1.67 µg/mL. These concentrations were used in subsequent experiments unless otherwise indicated.

Figure 2 Effect of anticoagulants on the dilute Russell viper venom time (DRVVT) in normal plasma. A, B, and C, Vertical point plots demonstrating the effect of anticoagulants on test/confirm ratios (see the “Materials and Methods” section). A, Heparin did not cause a prolongation in the test/confirm ratio owing to the presence of an antiheparin agent in the Gradipore DRVVT kit. B, Argatroban also did not increase the test/confirm ratio to values above the reference range (gray bar). C, However, lepirudin increased the test/confirm ratio to values above the reference range in all samples tested (\( P < .001 \)). D, E, and F, Vertical point plots demonstrating the effect of anticoagulants on percent correction of ratios (see the “Materials and Methods” section). Concordant with the test/confirm ratios, although heparin (D) and argatroban (E) did not prolong the percent correction of ratio, in 6 of 6 cases with lepirudin (F), the percent correction of ratio increased to values above the reference range (\( P < .001 \)). Reference ranges are represented as shaded areas and are as follows: test/confirm ratio, 0.94 to 1.17; percent correction of ratio, −10% to +15%.
In the presence of argatroban, however, the DRVVT ratio was increased (before, 0.94 ± 0.05; with argatroban, 2.88 ± 0.23; n = 6; P < .001; data not shown), suggesting the presence of an anticoagulant. Nevertheless, the test/confirm ratio (before, 1.0 ± 0.1; with argatroban, 1.0 ± 0.1; n = 6; P = .24) and the percent correction of ratio (before, 2.5% ± 3.8%; with argatroban, −0.5% ± 6.1%; n = 6; P = .25) [Figure 2E] were not significantly altered. Thus, argatroban added to normal plasma samples does not produce a pattern suggestive of an LA. Identical findings for the DRVVT ratio, percent correction of ratio, and test/confirm ratio were observed at higher doses of argatroban (2.9 µg/mL; data not shown), suggesting that these results would be observed throughout the therapeutic concentration range of argatroban in plasma.

Lepirudin increased not only the DRVVT ratio (before, 0.98 ± 0.07; with lepirudin, 2.77 ± 0.39; n = 6; P < .001; data not shown) but also the test/confirm ratio (before, 1.02 ± 0.05; with lepirudin, 1.34 ± 0.13; n = 6; P < .001) [Figure 2C] and the percent correction of ratio (before, −0.3% ± 4.7%; with lepirudin, 23.4% ± 6.5%; n = 6; P < .001) [Figure 2E]. Identical findings for the DRVVT ratio, percent correction of ratio, and test/confirm ratio also were observed at the much lower concentration of 0.4 µg/mL of lepirudin (data not shown). In other words, the addition of lepirudin to normal plasma samples consistently produced a test/confirm ratio and a percent correction of ratio greater than the upper limit of the reference range, resulting in values that would generate false-positive interpretations for the presence of an LA.

To determine whether direct thrombin inhibitors also might affect the interpretation of DRVVT testing in true LA-positive plasma samples, we added argatroban and lepirudin to samples of plasma that previously tested positive for an LA (see “Identification of Lupus-Positive Samples”). Heparin was not used in these experiments due to limited plasma volumes and the presence of an antiheparin agent in the Gradipore DRVVT kit. In 2 plasma samples, results of repeated DRVVTs performed after thawing were less than the threshold for positivity using the percent correction of ratio (>15%) [Figure 3C] but not the test/confirm ratio (>1.17) [Figure 3A]. These samples were included in this analysis because they met our initial inclusion criteria. The baseline aPTT in all thawed samples was 33.4 ± 7.6 seconds and increased to 86.5 ± 27.6 seconds with the addition of argatroban and 92.6 ± 34.1 seconds with the addition of lepirudin (data not shown).

In LA-positive samples, the DRVVT ratio again was increased in the presence of argatroban (before, 1.35 ± 0.24; with argatroban, 4.11 ± 0.94; n = 9; P < .001; data not shown), demonstrating the presence of an anticoagulant. Changes to the mean of the test/confirm ratio (before, 1.44 ± 0.22; with argatroban, 1.39 ± 0.25; n = 9; P = .11; Figure 3A) or the percent correction of ratio again were not significantly altered. Thus, argatroban added to normal plasma samples does not produce a pattern suggestive of an anticoagulant. Nevertheless, the test/confirm ratio (before, 1.39 ± 0.25; n = 9; P = .11), but in 2 of 9 samples, the percent correction of ratio decreased to values now within the reference range. Two additional samples had negative percent correction of ratios and remained negative in the presence of argatroban (see the “Discussion” section). Lepirudin increased the percent correction of ratio in 9 of 9 samples (P < .001). Reference ranges are represented as shaded areas and are as follows: test/confirm ratio, 0.94 to 1.17; and percent correction of ratio, −10% to +15%.

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of ratio (before, 20.9% ± 10.7%; with argatroban, 16.7% ± 14.2%; n = 9; P = .13; Figure 3C) did not reach statistical significance. It should be noted, however, that in 2 of the 7 samples that had initial percent correction of ratios of more than 15%, the percent correction of ratio subsequently fell below this threshold for positivity in the presence of argatroban (from 16.6% to 0.8% in one sample and from 20.3% to 9.2% in the other; Figure 3C). Two samples also dropped below the threshold for positivity using the test/confirm ratio (Figure 3A). Both of these latter samples were negative using the percent correction of ratio. Regardless of whether data are interpreted using the test/confirm ratio or the percent correction of ratio, argatroban seems to produce a false-negative result in some LA-positive samples.

When added to LA-positive plasma samples, lepirudin again resulted in a prolongation of the DRVVT ratio (before, 1.35 ± 0.24; with lepirudin, 5.22 ± 1.38; n = 9; P < .001; data not shown), the test/confirm ratio (before, 1.44 ± 0.22; with lepirudin, 2.13 ± 0.53; n = 9; P < .001; Figure 3B), and the percent correction of ratio (before, 20.9% ± 10.7%; with lepirudin, 44.2% ± 13.4%; n = 9; P < .001; Figure 3D).

Because the guidelines for diagnosis of an LA suggest testing for prolongation in 2 different phospholipid-dependent clotting assays, similar experiments were repeated using a PTT-LA reagent and platelet lysate as a source of high-concentration phospholipid. These experiments also used normal donor plasma and the previously diagnosed LA-positive samples. As expected, direct thrombin inhibitors dramatically prolonged the baseline PTT-LA in samples from healthy donors (control, 36.8 ± 1.8 seconds; with argatroban, 93.0 ± 7.4 seconds; with lepirudin, 95.6 ± 6.6 seconds; data not shown) and LA-positive samples (control, 44.7 ± 10.1 seconds; with argatroban, 115.9 ± 31.6 seconds; with lepirudin, 130.2 ± 41.9 seconds; data not shown). All 8 plasma samples from healthy donors had a baseline PTT-LA correction in the reference range (concentration, 0 µg/mL; average, 1.5 ± 0.6 seconds; upper limit of normal, 1 second). Only 4 of 9 prior LA-positive samples also had positive PTT-LA corrections (concentration, 0 µg/mL; average across all 9 samples, 2.4 ± 3.4 seconds), although it should be noted that 1 of these 5 negative samples also had a postthaw negative percent correction of ratio as seen in Figures 3C and 3D. The discrepancy between the DRVVT and PTT-LA emphasizes the critical importance of screening for LAs with multiple coagulation-based tests.

Both argatroban and lepirudin decreased the PTT-LA correction in samples of healthy donors (before, −1.5 ± 0.6 seconds; with argatroban, −6.0 ± 2.2 seconds; with lepirudin, −6.3 ± 2.1 seconds; n = 8; P < .001 for both; Figures 4A and 4B) (Figure 4E). In LA-positive samples, the PTT-LA corrections increased from an average of 2.4 ± 3.4 seconds before to 9.1 ± 13.6 seconds with argatroban and 12.1 ± 18.9 seconds with lepirudin, although these increases were not statistically significant, possibly owing to the small number of cases (P = .09 and P = .10 respectively; Figures 4C and 4D) (Figure 4F).

It should be noted that in 1 sample of LA plasma that had a negative PTT-LA correction at baseline, the PTT-LA correction increased to more than the threshold of positivity with the addition of argatroban or lepirudin (Figures 4C and 4D).

Discussion

The present study demonstrates that concentrations of direct thrombin inhibitors in plasma within the therapeutic range potentially can modify the results and interpretation of LA testing. With respect to the DRVVT, lepirudin prolonged the percent correction of ratio and test/confirm ratio in normal (Figures 2C and 2F) and LA-positive (Figures 3B and 3D) plasma samples. These results suggest that in the absence of complete a priori knowledge about the patient’s drug regimen, a DRVVT result might be interpreted as falsely positive in the presence of lepirudin. This information adds to a growing body of evidence that advocates particular caution in the interpretation of coagulation-based tests in the presence of direct thrombin inhibitors, especially in LA testing.23-25

Although argatroban did not affect the results of DRVVTS in plasma samples from healthy donors (Figures 2B and 2E), in samples of plasma from LA-positive patients, a small number of previously diagnosed DRVVT-positive samples were below the threshold in the presence of argatroban (Figures 3A and 3C). Together, these data suggest that interpretation of DRVVT data in the presence of any direct thrombin inhibitors potentially might lead to erroneous diagnoses of LA status.

When we used an LA-sensitive PTT reagent, argatroban and lepirudin dramatically increased the baseline PTT-LA. In view of this effect on the PTT-LA, simple mixing studies of patient and control plasma samples cannot distinguish between a prolonged result for the mixture due to an LA vs the presence of a direct thrombin inhibitor. In the PNP correction phase of the PTT-LA test system, however, argatroban and lepirudin caused a decrease in average PTT-LA corrections in plasma samples from healthy donors (Figures 4A, 4B, and 4E) but an upward trend (although not statistically significant) in the degree of PTT-LA corrections in plasma samples from LA-positive patients (Figures 4C, 4D, and 4F). Moreover, in 1 sample of LA-positive plasma (see the “Materials and Methods” section for inclusion criteria), a previously negative PTT-LA correction became positive.

The finding that the presence of direct thrombin inhibitors in the PTT-LA test widens the separation between LA-positive and LA-negative samples appears of particular interest because it raises the possibility that the sensitivity and specificity of this test system might be improved through inclusion of direct thrombin inhibitors in the assay. The apparent ability of lepirudin and argatroban to exaggerate the difference between the
absence and presence of an LA in the PTT-LA test is reminiscent of a similar exaggeration observed with increasing dilutions of tissue thromboplastin in the dilute tissue thromboplastin inhibition test. Further studies will be required to unravel the precise mechanisms underlying this intriguing effect.

The present report emphasizes the importance of knowing a patient’s anticoagulant drug regimen before interpreting the results of LA testing. The possibility that oral direct-thrombin inhibitors might one day replace warfarin-based anticoagulation makes these findings all the more relevant. Future studies will need to focus on whether these new classes of anticoagulants also might affect the results and interpretation of LA testing.

**Figure 4** Effect of anticoagulants on the lupus anticoagulant (LA)–sensitive activated partial thromboplastin time (PTT-LA) using the platelet neutralization procedure in LA-negative and positive plasma samples. A and B, Vertical point plots demonstrating the effect of anticoagulants on PTT-LA corrections (see the “Materials and Methods” section) in LA-negative plasma samples. PTT-LA corrections decreased from –1.5 ± 0.6 to –6.0 ± 2.2 seconds with argatroban (A) and –6.3 ± 2.1 seconds with lepirudin (B) (P < .001). C and D, Vertical point plots demonstrating the effect of anticoagulants on PTT-LA corrections in LA-positive plasma samples. PTT-LA corrections on average increased from 2.4 ± 3.4 seconds at baseline to 9.1 ± 13.6 seconds with argatroban (C) and 12.1 ± 18.9 seconds with lepirudin (D), although these increases did not reach statistical significance (P = .09 and P = .10, respectively). E and F, Summary histograms of the effects of argatroban (Arg) and lepirudin (Lep) in LA-negative plasma (E) and LA-positive plasma (F) samples. Threshold for positivity for the PTT-LA correction using neutralized platelets was 1 second (dashed line in A, B, C, and D; reference range, –2.5 to +1.0 seconds).

**References**


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