Dabigatran Inhibits Staphylococcus aureus Coagulase Activity

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The ability of Staphylococcus aureus to clot plasma through conformational activation of prothrombin by staphylocoagulase is used to distinguish S. aureus from coagulase-negative staphylococci. We show that while the direct thrombin inhibitor dabigatran inhibits staphylocoagulase activity, the clinical use of dabigatran etexilate is not expected to interfere with direct tube coagulase testing.

Since the early notion, developed more than a century ago, that the ability to clot plasma correlates with the pathogenic potential of staphylococci (3, 14), this phenomenon has been used to distinguish Staphylococcus aureus from less virulent, coagulase-negative staphylococci. Although the use of new molecular methods for rapid identification of S. aureus from blood culture bottles is expanding (15, 17), detection of coagulase activity by direct tube coagulase (DTC) testing remains a cost-effective, easy to perform, and reliable method for early detection of S. aureus in blood culture broth with a Gram stain result suggestive of staphylococci (10, 13, 16, 23). Since false-negative results increase the therapeutic delay, the carryover of contaminants from either the culture broth or the patient’s plasma that interfere with the tube test clotting reaction has to be avoided (23).

Clotting occurs when fibrinogen is converted into fibrin by thrombin. Under physiological conditions, the proteolytic activation of the inactive precursor prothrombin into thrombin is the final step in the tightly regulated coagulation cascade. Staphylocoagulase secreted by S. aureus directly binds to prothrombin to form the staphylothrombin complex and thus bypasses the coagulation cascade and its physiological regulation (6). As a consequence of this unique nonproteolytic direct thrombin activation, treatment with heparins, vitamin K antagonists, or the thrombin inhibitor hirudin does not interfere with staphylothrombin function (9) and is of no concern for DTC testing.

In view of the recent availability and increasing clinical use of the new direct thrombin inhibitor dabigatran etexilate, we studied whether dabigatran inhibits staphylocoagulase and whether clinical use of dabigatran etexilate is expected to interfere with DTC testing.

To test these possibilities, we measured coagulation of citrated human plasma as the change in absorbance at 405 nm (A405) after the addition of purified staphylocoagulase (3 nM) in the presence of increasing concentrations (0, 0.625, 1.25, 2.5, 5, and 10 nM) of dabigatran (Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany). Coagulase (Synapse BV, Maastricht, Netherlands) was purified over a bovine thrombin column as described elsewhere (2). To compare the pharmacokinetics of the inhibition of activated human thrombin and the staphylothrombin complex, we added the chromogene substrate Biophen CS-01(38) (Hyphen BioMed, Neuville-sur-Oise, France) to microwells containing dabigatran and either purified human alpha-thrombin (Enzyme Research Laboratories, Swansea, United Kingdom) or a mixture of staphylocoagulase and purified human prothrombin (Enzyme Research Laboratories, Swansea, United Kingdom). We measured the maximum substrate conversion rate (change in A405) in the presence of a range of dabigatran concentrations (0, 100, 200, 400, 800, and 1,600 nM) at 4 different substrate concentrations (100, 200, 400, and 800 μM) and determined the Ks of dabigatran for both alpha-thrombin and the staphylothrombin complex from the resulting substrate-velocity curves.

To evaluate the effect of dabigatran on tube coagulase testing, we obtained 25 consecutive blood culture samples in BacT/Alert bottles (bioMérieux) that had tested positive for S. aureus from the University of Leuven hospital laboratory. DTC testing was performed as follows: we mixed 100 μl of the culture broth with 300 μl of EDTA-treated rabbit plasma (Remel, Lenexa, KS), which is used routinely as a coagulase tube test reagent and had been spiked with dabigatran (final concentrations of 200, 100, 50, 25, 12.5, and 6.25 nM), and assessed clotting after 1, 2, and 4 h of aerobic incubation at 37°C.

In order to further determine the impact of the clinical use of dabigatran etexilate on DTC testing in routine practice, we inoculated 12 aerobic blood culture bottles (BacT/Alert; bioMérieux) containing either 10 ml of citrated blood (6 bottles) or 10 ml of blood spiked with dabigatran (concentration in plasma, 400 nM; 6 bottles) with a small inoculum of a reference S. aureus strain (ATCC 25923). After inoculation, the culture bottles were incubated in an automated BacT/Alert three-dimensional microbial detection system (bioMérieux). When culture samples were detected to be positive, 5 separate DTC tests for each culture bottle were performed by mixing 100 μl of culture broth with 300 μl of rabbit plasma (Remel, Lenexa, KS) and assessing coagulation after 1, 2, and 4 h of aerobic incubation.

We found that dabigatran delays clotting of human plasma
Our data demonstrate that dabigatran indeed inhibits the clotting of rabbit plasma induced by staphylocoagulase in a time- and concentration-dependent manner and that this inhibition can result in false-negative or delayed-positive tube test readouts. However, dabigatran concentrations of 25 nM and below in a test tube did not affect the final readout in our experiments. Clinically relevant peak concentrations of dabigatran in plasma are ~180 ng/ml (~350 nM) (21, 22). For DTC testing, the patient blood sample is first diluted 1/5 in the blood culture medium and then diluted again 1/3 to 1/4 when the culture broth is added to the rabbit plasma. Thus, considering that the blood sample is diluted a minimum of 1/15 in the test tube, dabigatran concentrations in the clinical setting are expected to be well below this 25 nM threshold and the clinical use of dabigatran etexilate is not expected to affect the readout after 4 h according to our observations.

To confirm this, we inoculated blood with a supratherapeutic dabigatran concentration (400 nM) and a reference S. aureus strain and performed standard DTC testing when the culture bottles were detected as positive. Despite the high dabigatran concentration used, there was no change in the coagulase test readouts compared to those for the control culture bottles without dabigatran. Taken together, our data show that while dabigatran inhibits staphylothrombin at clinically relevant concentrations in plasma, concentrations obtained after dilution in blood culture broth and coagulase reagent do not affect DTC testing.

The availability of a pharmacological staphylothrombin inhibitor could facilitate further research on the controversial role of staphylocoagulase in the pathogenesis of S. aureus infections. Although staphylocoagulase is classically considered to be a virulence factor, the available evidence from animal infection models is conflicting. Some studies showed decreased virulence of coagulase-negative mutants (1, 7, 8, 12, 20), but these mutant strains arose from nonspecific increased mutagenesis and probably had inherently reduced virulence compared to the nonmutant parent strain. Coagulase-negative mutants obtained by site-specific mutagenesis showed reduced virulence in a murine hematogenous pulmonary infection model (18), and inhibition of coagulase activity using inhibitory RNA reduced bacterial loads in an animal S. aureus bacteremia model (24), showing the potential of coagulase inhibition strategies in these settings. However, such studies either require the use of mutant strains or involve laborious

### Table 1. Effect of dabigatran on direct tube coagulase testing

<table>
<thead>
<tr>
<th>Dabigatran (nM)</th>
<th>1 h (%) of false-negative tube test readouts</th>
<th>2 h (%)</th>
<th>4 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>13 (52)</td>
<td>15 (60)</td>
<td>8 (32)</td>
</tr>
<tr>
<td>100</td>
<td>13 (52)</td>
<td>13 (52)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>50</td>
<td>8 (32)</td>
<td>6 (24)</td>
<td>1 (4)</td>
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<td>25</td>
<td>2 (8)</td>
<td>2 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>12.5</td>
<td>1 (4)</td>
<td></td>
<td>0 (0)</td>
</tr>
<tr>
<td>6.25</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*The table shows numbers and percentages of direct tube coagulase tests with a negative readout as opposed to a positive control for a range of dabigatran concentrations. Results are shown for readouts at 1, 2, and 4 h for all tubes (n = 25).*
strategies to inhibit coagulase activity that are not applicable in a clinical setting.

In conclusion, we show that dabigatran is a potent inhibitor of staphylorthrombin, offering new opportunities to study the role of staphylocoagulase as a virulence factor and the impact of its inhibition in S. aureus clinical infections. Although dabigatran also inhibits the clotting of rabbit plasma by staphylothrombin, its expanding clinical use is not anticipated to be of concern for diagnostic tube testing.

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REFERENCES