Prophylaxis of Experimental Endocarditis With Antiplatelet and Antithrombin Agents: A Role for Long-term Prevention of Infective Endocarditis in Humans?

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**Background.** Infective endocarditis (IE) mostly occurs after spontaneous low-grade bacteremia. Thus, IE cannot be prevented by circumstantial antibiotic prophylaxis. Platelet activation following bacterial-fibrinogen interaction or thrombin-mediated fibrinogen-fibrin polymerization is a critical step in vegetation formation. We tested the efficacy of antiplatelet and antithrombin to prevent experimental IE.

**Methods.** A rat model of experimental IE following prolonged low-grade bacteremia mimicking smoldering bacteremia in humans was used. Prophylaxis with antiplatelets (aspirin, ticlopidine [alone or in combination], eptifibatide, or abciximab) or anticoagulants (antithrombin dabigatran etexilate or anti–vitamin K acenocoumarol) was started 2 days before inoculation with *Streptococcus gordonii* or *Staphylococcus aureus*. Valve infection was assessed 24 hours later.

**Results.** Aspirin plus ticlopidine, as well as abciximab, protected 45%–88% of animals against *S. gordonii* and *S. aureus* IE (*P* < .05). Dabigatran etexilate protected 75% of rats against IE due to *S. aureus* (*P* < .005) but failed to protect against *S. gordonii* (<30% protection). Acenocoumarol was ineffective.

**Conclusions.** Antiplatelet and direct antithrombin agents may be useful in the prophylaxis of IE in humans. In particular, the potential dual benefit of dabigatran etexilate might be reconsidered for patients with prosthetic valves, who require life-long anticoagulation and in whom *S. aureus* IE is associated with high mortality.

**Keywords.** experimental endocarditis; prophylaxis; antiplatelets; anticoagulants; *Staphylococcus aureus*; *Streptococcus gordonii*.
bacteremia occurring during normal day-to-day activities. This assumption was further supported by the fact that the cumulative numbers of bacteria circulating in the blood during chewing or toothbrushing are vastly superior (by $10^{5-6}$ times) than the numbers resulting from punctual dental procedures [5, 6].

On this basis, the American Heart Association and the European Society for Cardiology have revisited their guidelines for IE prophylaxis and drastically decreased the indications for prophylactic antibiotic use [7, 8]. However, while restricting the spectrum of antibiotic prophylaxis, the new guidelines do not propose any alternatives to prevent IE caused by the numerous occurrences of spontaneous bacteremia.

Because IE is life-threatening and difficult to treat, general protective strategies are desired for high-risk patients [3, 9]. Such measures should be compatible with chronic use and based on validated physiopathological concepts. Here we tested the hypothesis that interfering with bacteria-induced platelet aggregation or prothrombin activation could fit this purpose. The logic of this approach was based on the fact that bacteria capable of interacting with platelets are more prone to induce IE [10].

Most cases of IE are due to oral streptococci (eg, *Streptococcus gordonii*) or *Staphylococcus aureus* [2]. We tested several antiplatelet and anticoagulant drugs for their efficacy in the prophylaxis of experimental IE induced by *S. gordonii* and *S. aureus*. Antiplatelet drugs included aspirin, ticlopidine, eptifibatide, and abciximab. Aspirin inhibits platelet cyclooxygenase 1 (COX1) activity, thereby blocking thromboxane A$_2$ (TXA$_2$), resulting in a reduction of platelet activation. Ticlopidine inhibits the platelet ADP receptor, P$_2$Y$_{12}$, thereby suppressing ADP-induced platelet activation and aggregation. Both TXA$_2$ and ADP are major agonists of platelet aggregation. Eptifibatide and abciximab act by antagonizing the GP IIb/IIIa receptor for fibrinogen on the platelet surface. These drugs block platelet aggregation by preventing the binding of fibrinogen molecules that cross-link adjacent platelets [11]. Anticoagulants included dabigatran etexilate and acenocoumarol. Dabigatran etexilate competitively binds to the active site of thrombin, preventing the conversion of soluble fibrinogen to insoluble fibrin and subsequent clot formation. Acenocoumarol exhibited its anticoagulant effect by inhibition of vitamin K epoxide reductase, ultimately leading to the depletion of functional vitamin K–dependent clotting factors [12].

Bacteria were inoculated continuously at low concentrations to simulate the constant exposure to spontaneous smoldering bacteremia occurring most of the time in humans during routine daily activities, in the case of oral streptococci [6], or from more or less prolonged low-grade discharges from an infected intravascular device, in the case of staphylococci [13]. The results indicate that interfering with platelet activation is an effective way to prevent valve infection against these 2 pathogens.

### MATERIALS AND METHODS

#### Ethics Statement

Blood specimens were obtained from one of the authors (T. R. V.) under safe conditions. Ethical approval was not required. The animal studies were performed in strict accordance with the recommendations of the Swiss Federal Act on Animal Protection. All animal protocols were reviewed and approved by the Cantonal Committee on Animal Experiments of the State of Vaud (permit 879.8). A mixture of ketamine (75 mg/kg) and midazolam (5 mg/kg) anesthesia was administered to animals before any surgical procedure. During all animal experimentation procedures, all efforts were made to minimize the potential for suffering.

#### Bacterial Strains and Growth Conditions

The well-described isolates of *S. gordonii* (strain Challi) and coagulase-positive *S. aureus* (strain Newman) [14] were used. *S. gordonii* was grown at 37°C with 5% CO$_2$ in brain-heart infusion broth (Difco; Becton Dickinson, Sparks, MD) and midazolam (5 mg/kg) anesthesia was administered to animals before any surgical procedure. During all animal experimentation procedures, all efforts were made to minimize the potential for suffering.

#### Antiplatelet and Anticoagulant Compounds

Antiplatelet drugs included aspirin (Aspegic, Sanofi Aventis, Meyrin/Genève, Switzerland), ticlopidine (Sigma-Aldrich, Buchs, Switzerland), eptifibatide (Integrilin, GlaxoSmithKline, Münchenbuchsee, Switzerland), and abciximab (ReoPro, Eli Lilly, Vernier/Genève, Switzerland). Anticoagulants included dabigatran etexilate (Pradaxa, Boehringer Ingelheim, Basel, Switzerland) and acenocoumarol (Sigma-Aldrich). Dabigatran, the active form of dabigatran etexilate, was kindly provided by Dr Peter Verhamme (Leuven, Belgium).

None of these agents possessed antimicrobial activity against the bacterial test strains, as shown by minimal inhibitory concentrations of ≥128 µg/mL, far in excess of pharmacologically relevant concentrations. Moreover, after 24 hours of exposure of $10^6$ colony-forming units (CFU)/mL of the organisms to either 50 µg/mL of aspirin, 2.0 µg/mL of ticlopidine, 2.5 µg/mL of eptifibatide, 10 µg/mL of abciximab, 150 ng/mL of dabigatran, or 2 µg/mL of acenocoumarol (ie, concentrations within the therapeutic plasma levels in humans after standard doses [15–20]), no effect on bacterial growth rates was observed.

#### Assessment of Drug Effect on Bacteria-Induced Platelet Aggregation and Plasma Coagulation

Platelet-rich plasma (PRP) and platelet-poor plasma for platelet-aggregation tests were obtained from anticoagulated human blood as described previously [21]. The extent of platelet aggregation inhibition of aspirin, ticlopidine, aspirin plus ticlopidine, eptifibatide, and abciximab was performed by light transmission ($A_{600\text{ nm}}$), using a fluorometer Infinite 200 Pro (Tecan, Salzburg, Austria) [21]. Platelet aggregation was monitored every minute for 20 minutes. The various inhibitors were
compared with regard to the interval between the addition of bacteria to the PRP suspension and the onset of the aggregation response (lag time). The light transmission of PRP alone was defined as 0% aggregation. Platelet aggregation with ADP (10 μM) was used as a positive control. Three independent assays were performed.

The anticoagulant effect of dabigatran etexilate and acenocoumarol was assessed by a modification of the clotting time test described by Cheng et al. In brief, blood of control rats and rats receiving anticoagulants as in in vivo prophylactic experiments was drawn into plastic tubes without any anticoagulant and allowed to clot. In some assays, S. gordonii, S. aureus, or saline were added to the blood to test their possible interference with coagulation. In these experiments, 500 μL of rat blood was spiked with bacteria (final concentration, 10^5 CFU/mL), and tubes were observed until visible blood clotting appeared.

**Rat Endocarditis Model and Prophylaxis Study**

The production of catheter-induced aortic vegetations and the installation of a permanent intravenous line connected to a programmable infusion-pump device were performed in female Wistar rats, as described previously.

Prophylaxis began after the insertion of the intracardiac catheter and lasted for 48 hours. Aspirin (8 mg/kg) and ticlopidine (10 mg/kg), alone or in combination, were administered by an intravenous bolus injection every 12 hours. Eptifibatide was administered by a bolus of 180 μg/kg followed by a continuous infusion of 2 μg/kg/minute over 24 hours. Abciximab was given every 12 hours by a bolus of 0.25 mg/kg followed by a continuous infusion of 0.125 μg/kg/minute. Dabigatran etexilate prophylaxis was performed by intraperitoneal injections of 5 or 10 mg/kg every 12 hours. Two different doses were tested because dabigatran etexilate has been shown to inhibit thrombin in a dose-dependent manner. For intraperitoneal injections of dabigatran etexilate, commercially available capsules of Pradaxa were dissolved as described. A solution of acenocoumarol (10 mg/mL) was prepared in dimethyl sulfoxide (Sigma-Aldrich) and adjusted to the desired concentration in a 1:1 (vol/vol) ratio of saline to distilled water. The chosen doses were previously used in animal models or in humans. Control rats received saline. Pharmacokinetics analysis has not been attempted.

Forty-eight hours after starting prophylaxis, animals were inoculated with 10^9 CFU of S. gordonii or 10^6 CFU of S. aureus. The inocula were prepared by diluted overnight cultures until the desired concentration and progressively delivered at a pace of 0.0017 mL/minute over 10 hours. Rats were euthanized 24 hours after inoculation. The cardiac vegetations were sterilely removed, weighed, and processed as described elsewhere to determine the number of viable organisms.

**Statistical Analysis**

The quantification of platelet aggregation inhibition (lag times), clotting times, and the weights of the vegetations were expressed as mean ± standard deviation (SD) and compared by the Student t test. The percentage of infected vegetations was analyzed by the Fisher exact test. All statistical analyses were performed with GraphPad Prism software (version 4.0 for Windows; GraphPad Software, La Jolla, CA: http://www.graphpad.com). Differences were considered significant at P values of <.05, by use of 2-sided significance levels.

**RESULTS**

**Platelet Aggregation**

One key question was the platelet aggregation phenotype in the presence or absence of bacteria or antiaggregants. In the absence of either bacteria or drug, the lag time for spontaneous platelet aggregation was >20 minutes, whereas ADP induced aggregation within 1 minute. When S. gordonii and S. aureus alone were added to platelets, the mean lag times (±SD) of platelet aggregation were 9.0 ± 0.6 minutes and 1.3 ± 0.7 minutes, respectively, underlining the superior aggregation capacity of S. aureus. In the presence of S. gordonii, all of the antiaggregants prolonged the lag time of microbe-induced aggregation to >20 minutes (upper measurement limit). In the presence of S. aureus, aspirin alone, ticlopidine alone, or aspirin and ticlopidine in combination significantly increased the lag time (from 1 to approximately 5 minutes). With regard to GPIIb/IIIa inhibitors, both eptifibatide and abciximab completely inhibited S. aureus–induced platelet aggregation (no platelet aggregation was observed within 20 minutes).

**Prevention of Experimental IE Due to S. gordonii by Inhibitors of Platelet Activation**

The efficacy of antiplatelet drugs to prevent experimental S. gordonii IE is depicted in Figure 1. In control rats, the incidence of valve infection was 78%. Compared with these control values, aspirin or ticlopidine alone failed to prevent IE (87% and 80% of vegetations infected, respectively). In contrast, the combination of aspirin and ticlopidine effectively prevented IE (21% of vegetations infected; P = .0007).

Eptifibatide failed to prevent IE (75% of vegetations infected), whereas abciximab successfully prevented IE (15% of vegetations infected; P = .0003). Bleeding events were observed in 2 of 12 animals receiving eptifibatide and in any animals receiving aspirin-ticlopidine or abciximab.

**Prevention of Experimental IE Due to S. aureus by Inhibitors of Platelet Activation**

Figure 2 depicts a similar experiment performed with S. aureus. In control rats, the incidence of IE was 93%. Aspirin or ticlopidine alone failed to prevent IE (100% and 83% vegetations infected;
infected, respectively). In contrast, aspirin combined with ticlopidine had a marginal but significant protective effect (55% of vegetations infected; \( P = .04 \)). Eptifibatide failed completely (100% of vegetations infected), yet bleeding events occurred in 4 of 7 animals, indicating that the drug indeed affected platelet activity. In sharp contrast, abciximab was highly successful in preventing IE (12% of vegetations infected; \( P = .0002 \)), and no bleeding events were observed.

**Prevention of Experimental Endocarditis Due to \textit{S. gordonii} by Antithrombin and Anti–Vitamin K Agents**

The results shown in Figure 1 and Figure 2 highlight the prophylactic efficacy of the anti-GPIIb/IIIa monoclonal antibody abciximab. As abciximab is supposed to block the fibrinogen-fibrin platelet interaction in the fibrinogen-fibrin platelet network, we investigated whether interfering with the upstream bacterial-fibrinogen binding by use of dabigatran etexilate or acenocoumarol would have the same effect. Dabigatran etexilate at either 5 mg/kg or 10 mg/kg failed to prevent experimental \textit{S. gordonii} IE (100% and 70% of vegetations infected, respectively; Figure 3). Bleeding events were observed in only 1 of 10 animals with the 10 mg/kg dose. Likewise, acenocoumarol did not successfully prevent IE (67% of vegetations infected), and bleeding complications were observed in 2 of 6 animals. Both dabigatran etexilate and acenocoumarol significantly increased blood clotting time both in the absence or the presence of \textit{S. gordonii} (Table 1), demonstrating that they were biologically active in the animals.

**Prevention of Experimental Endocarditis Due to \textit{S. aureus} by Antithrombin and Anti–Vitamin K Agents**

Figure 4 shows the results of prophylaxis with \textit{S. aureus}. At a dose of 5 mg/kg, dabigatran etexilate significantly decreased the rate of IE (44% of vegetations infected; \( P = .028 \)). There was no bleeding observed at this dose. Since dabigatran etexilate has a dose-dependent activity [26], we further tested its efficacy at 10 mg/kg. At this dose, dabigatran etexilate decreased the rate of IE (25% of vegetations infected; \( P = .003 \)), but bleeding events were observed in 2 of 8 animals. In contrast, acenocoumarol failed to prevent IE (100% of vegetations infected), and bleeding events were observed in 4 of 10 animals.

The anticoagulant effect of these compounds in animals was demonstrated by a significant increase in the blood clotting time both in the absence or presence of bacteria (Table 1).

**DISCUSSION**

In the present study, we observed a significant protection against experimental \textit{S. gordonii} or \textit{S. aureus} IE by drugs...
interfering with platelet activation/aggregation, including the combination of aspirin plus ticlopidine, abciximab, and the antithrombin dabigatran etexilate.

Aspirin (a COX1 inhibitor) and ticlopidine (an ADP receptor P₂Y₁₂ inhibitor) block the effector signal transduction leading to platelet activation and aggregation [11]. This blockage occurs downstream of the activator signals transmitted by interactions of S. gordonii or S. aureus with the agonist platelet receptors GPIb, GPVI, or GPIIb/IIIa [28]. Thus, aspirin and ticlopidine nonspecifically prevented platelet activation/aggregation against both organisms by disrupting the platelet-platelet interaction. The prophylactic effect of the aspirin-ticlopidine combination was previously tested in the rabbit model of S. aureus IE by Nicolau et al [29]. These authors reported a statistically significant decrease in mean vegetation weights in treated animals but no decrease in valve infections. In contrast, the present results showed a decrease in both vegetation weights and valve infections. We postulate that this difference is due to the inoculation technique used in classical models of experimental IE, in which animals are infected with intravenous bolus injections of high bacterial numbers. This results in transient (1–2 minutes) bacteremia of >1000 CFU/mL, which may overcome the beneficial effect of antplatelet prophylaxis. In contrast, the more realistic model used here produced bacterial concentrations in the blood that did not exceed 2–50 CFU/mL but persisted for a prolonged period [14]. These bacterial concentrations in the blood are

Table 1. Anticoagulant Effect of Dabigatran Etxetilate and Acenocoumarol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clotting Time, min, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td>No drug</td>
<td>6.00 ± 0.40</td>
</tr>
<tr>
<td>Dabigatran etexilate</td>
<td>40.75 ± 2.13a</td>
</tr>
<tr>
<td>Acenocoumarol</td>
<td>24.25 ± 2.83a</td>
</tr>
</tbody>
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The anticoagulant effect of dabigatran etexilate and acenocoumarol was assessed in 3 independent experiments by measuring the clotting time in the blood of rats 48 hours after the onset of the prophylactic regimen.

*P < .02, by the Student t test, compared with the clotting time in the absence of drug (no drug).
comparable to those measured after dental extraction in humans [5, 6] but are low enough to benefit from antiplatelet prophylaxis. Indeed, the difference between successful aspirin ticlopidine prophylaxis in the setting of continuous low-grade S. gordonii bacteremia versus transient high-grade bacteremia was substantiated in a parallel study in which we showed that this combination did not reduce the incidence of IE in the bolus inoculation model (data not shown).

Our results also showed that blocking the agonist GPIIb/IIIa fibrinogen platelet receptor with abciximab [11] afforded protective protection against both organisms, as well. On the other hand, eptifibatide, which targets the same receptor, failed completely. These opposite in vivo results, which occurred despite the fact that both drugs produced a similar antiaggregation effect (lag time) in vitro, are most likely explained by different pharmacokinetics and/or different affinity profiles for the GPIIb/IIIa platelet receptor [30]. Eptifibatide is a small molecule with a rapid rate of dissociation from GPIIb/IIIa and could be rapidly cleared from the system. In contrast, abciximab is a chimeric Fab fragment monoclonal antibody with greater, long-lasting affinity for GPIIb/IIIa that almost irreversibly inhibits the receptor.

S. aureus platelet activation/aggregation occurring via GPIIb/IIIa depends also on the presence of specific antibodies and the low-affinity immunoglobulin G (IgG) platelet receptor FcyRIIa. For example, anti-ClfA IgG serves as transduction signal for S. aureus platelet activation, together with a ClfA fibrinogen-fibrin bridge with GPIIb/IIIa [31]. However, rat platelets do not express FcyRIIa on their surface, in contrast to human platelets. This could affect the interaction of S. aureus with rat platelets and influence the effect of abciximab. However, the presence of FcyRIIa on human platelets should not affect the activity of this compound against S. aureus in humans. Indeed, even if S. aureus is recognized by anti-ClfA IgG and is attached to FcyRIIa, abciximab will still be able to block GPIIb/IIIa and subsequent bacterial-induced platelet activation/aggregation, because both FcyRIIa and GPIIb/IIIa are necessary to trigger it [31]. In the case of S. gordonii, the interaction with GPIIb/IIIa platelet receptor is mediated by PadA without specific IgG intervention and independently of the FcyRIIa receptor [31], and thus the effect of abciximab should be similar in rats and in humans.

We next tested the antithrombin dabigatran etexilate, which inhibits fibrinogen-fibrin polymerization promoted by staphylococcal coagulase. Staphylococcal coagulase binds to prothrombin to form a proteolytically active complex (staphylothrombin) that converts fibrinogen into fibrin and facilitates S. aureus-induced platelet aggregation [32]. Moreover, since dabigatran etexilate is an anticoagulant, we used acenocoumarol (a vitamin K antagonist) as a control. Dabigatran etexilate successfully prevented experimental IE due to S. aureus but not due to S. gordonii, while acenocoumarol had no effect against any tested bacteria. Protection of dabigatran etexilate against S. aureus experimental IE could be associated with the inhibition of fibrinogen-fibrin polymerization by blocking not only thrombin but also staphylothrombin, which exacerbates fibrin formation [33]. This would decrease platelet–platelet interaction occurring via GPIIb/IIIa receptor and, likely, also S. aureus–platelet interaction in humans. In the case of S. gordonii, the lack of dabigatran etexilate activity could be explained by the fact that this microorganism is able to induce fibrinogen-fibrin polymerization in a thrombin-independent way, using the FSS2 chalasin protein [34].

Taken together, the present results provide clues about how an antiplatelet or anticoagulant compound could play a role in the prevention of IE in humans, in addition to the known effect of such agents on the thrombotic-coagulation system. The principal mechanism appeared to be inhibition of platelet activation, not anticoagulation, as shown by the absence of efficacy of acenocoumarol.

Nevertheless, we cannot exclude that other additional effects of the drugs, outside of targeting platelet receptors, could contribute to protect animals from endocarditis. For instance, aspirin may also have a COX1-independent vitamin K antagonism [35]. Moreover, the ability of aspirin to protect the endothelium from oxidative stress, as well as its demonstrated capacity to alter the expression of S. aureus virulence factors [36], could also improve its efficacy. P2Y12 inhibitors as well as GPIIb/IIIa blockers, such as abciximab, may also have an antiinflammatory effect [37, 38]. Finally, dabigatran etexilate, by blocking thrombin, reduced thrombin-induced production of extracellular matrix proteins and proinflammatory factors [39]. Further investigation is required to clarify the role of these additional effects in our experimental model.

The question arises as to which antiplatelet or anticoagulant drug would be the most adequate for the prevention of IE in humans. The advantage of the combination of aspirin with ticlopidine (or equivalent newer molecules) over abciximab is that they can be administered orally for prolonged periods to decrease the risk of both streptococcal and staphylococcal IE simultaneously. In contrast, abciximab must be administered intravenously and serves essentially as a proof of concept unless user-friendly oral formulations are developed.

Long-term use of aspirin to prevent IE-related embolism has been a matter of debate [40]. Some authors reported no decrease in embolic events at the expense of a greater risk of bleeding [41], whereas others reported a beneficial effect [42]. On this basis, Eisen and Bayer [40] argued that antiplatelet prevention of IE should be reevaluated, especially with regard to its most severe form due to S. aureus, which is responsible for up to 50% of mortality in cases of prosthetic valve IE [9, 43].

Here we also underlined the high preventive efficacy of dabigatran etexilate against S. aureus infection and propose it as an alternative to anti–vitamin K compounds for anticoagulation in
patients with prosthetic valves because it would additionally protect from S. aureus infection. However, a phase 2 study comparing dabigatran etexilate to warfarin in prosthetic valve anticoagulation [44] was prematurely stopped because of an increase in thromboembolic events in the dabigatran etexilate group (http://www.fda.gov/drugs/drugsafety/ucm332912.htm). Detailed information on these results will be critical before such compounds can be definitively excluded from the prophylactic armamentarium.

In conclusion, our study demonstrates the beneficial effect of both antiplatelet and direct antithrombin drugs in the prevention of experimental endocarditis in a rat model mimicking spontaneous low-grade bacteremia in humans. These findings might have implications in the prophylaxis of IE in patients with at-risk valve lesions. As suggested by Eisen and Bayer [40], it might be time to reevaluate the beneficial effect of these drugs for prevention rather than treatment of IE.

Notes

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