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Antithrombotic Drug Interactions

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1. Introduction

Despite recent major pharmacological and device advances, percutaneous coronary intervention (PCI) remains a costly procedure with significant peri-procedural risk. Heparin has maintained the foundation of procedural anticoagulation, but heparin anticoagulation is unpredictable because it is an indirect thrombin inhibitor, which requires heparin cofactor II-antithrombin for its actions. Antithrombin levels vary widely in patients. In addition, the heparin-antithrombin complex is too large to inhibit clot-bound or fibrin-bound thrombin. Clinical functions can alter antithrombin levels, which further reduce heparin’s predictability. Heparin can also be inhibited by plasma proteins. These complexities can lead to both excessive anticoagulation with clinical bleeding and subtherapeutic heparinization with clinical coronary occlusion. Finally, heparin is associated with a 1–3% incidence of heparin-induced thrombocytopenia (HIT), which carries an increased risk for acute, subacute, and chronic thrombotic occlusion. The major pharmacological improvement in platelet efficacy has been the addition of glycoprotein IIb/IIIa inhibitors for PCIs. However, all GPIIb/IIIa inhibitors are associated with a risk of bleeding. Reduction of heparin doses has resulted in less clinical bleeding, but bleeding still occurs in a significant number of patients.

Investigations with direct thrombin inhibitors have been undertaken for both non-ST-elevation myocardial infarction (MI) and PCI. Hirudin has shown a small but definite reduction in coronary ischemia compared to heparin in acute coronary syndromes (ACS), but hirudin is associated with a statistically significant increase in the frequency of major bleeding. The modifications in the
hirudin molecule have resulted in bivalirudin, which has been tested in >4000 PCI patients compared to heparin. Bivalirudin showed a significant reduction in major bleeding (13.0% vs 9.89%, p<0.001) compared to heparin, indicating a trend toward fewer ischemic complications. Argatroban is smallest of the direct thrombin inhibitors, and has similar pharmacodynamics to bivalirudin (reversible, short half-life). A large body of evidence suggests that direct thrombin inhibitors (hirudin and bivalirudin) are more efficacious than heparin for treatment of ACS and that the small molecules—short half-life, reversible thrombin inhibitors (bivalirudin and argatroban)—are safer than heparin. The improved efficacy of argatroban should reduce the need for adjuvant GPIIb/IIIa inhibition, and therefore reduce bleeding as well as pharmacologic costs.

Argatroban (Novastan), a direct thrombin inhibitor, is a carboxy acid derivative that belongs to a class of peptidomimetics that also includes inogatran, efegatran, and napsagatran. Argatroban has now been approved in the United States as an alternative to heparin in patients with HIT. It binds covalently to the active site of thrombin (1). Argatroban was used in one trial of 50 patients with HIT who were undergoing plasma thromboplastin component antecedent percutaneous transluminal coronary angioplasty (PTCA) at a dose of 350 \( \mu \text{g/kg} \) bolus, and yielded encouraging results.

Reperfusion therapy of acute myocardial infarction (AMI) to establish reperfusion as quickly as possible is of primary importance. The use of fibrinolytics in combination with low molecular weight heparins (LMWHs) have provided encouraging results in randomized clinical trials. The encouraging results seen with LMWHs instead of unfractionated heparin (UFH) represent a definitive advancement in the field. If LMWHs are exerting their effects mainly their enhanced anti-Xa inhibition, then more specific and direct factor Xa inhibitors may be advantageous (4). The GPIIb/IIIa inhibitors can be used in combination with the thrombolytic agents in patients with AMI. Activase™ (alteplase, recombinant) in combination with GPIIb/IIIa inhibitors or TNKase in combination with GPIIIb/IIIa inhibitors can be used in patients with AMI.

The thrombi in the coronary arteries that cause AMI comprise of a platelet core in a fibrin-thrombin matrix. Following successful thrombolysis, re-occlusion is caused by excessive platelet activation, which makes the thrombi difficult to lyse. In these situations, adjunctive use of thrombolytic agents with GPIIb/IIIa inhibitors will prevent platelet activation and aggregation (5). Platelets bind to the walls of the vessel by attachment at Ia or Ib receptors on the platelet surface. Platelet-platelet binding is a result of interaction between GPIIb/IIIa receptors involving the fibrinogen and vWF (6).

Gold et al. have demonstrated that the platelet Fab fragment of the murine antibody 7E3-F(ab)2 to GPIIb/IIIa binds tightly to the GPIIb/IIIa receptor and inhibits platelet aggregation (7). In the TAMI-8, a non-randomized multici-
ter pilot study, 60 patients with AMI were given Activase with varied abciximab dosages of 0.1, 0.15, 0.20, and 0.25 mg/kg given at 3, 6, and 15 h after a 100-mg dose of Activase administered over a period of 3 h. Despite limitations of the study being small and not blinded, the safety profile was similar in the abciximab and control groups. However, in abciximab-treated patients, fewer major bleeding events, decreased recurrent ischemic events, and better coronary-artery patency, as evaluated by angiography, were observed.

An ongoing, double-blind, randomized, placebo-controlled, crossover trial of abciximab alone or in combination with low-dose Activase is being carried out in 26 patients with AMI. Each patient presented within 6 h of symptom onset with ST-segment elevation. Patients were initially given aspirin and heparin, and then randomized to receive either abciximab 0.25 mg/kg bolus or placebo followed by an angiogram 60–90 min later. Patients were crossed over and given the opposite treatment. A second angiogram was taken 10 min later. The results of the second angiogram, in which patients received abciximab alone, showed that eight patients had thrombolysis in myocardial infarction (TIMI) grade 0 flow, five patients had TIMI grade 1 flow, five patients had TIMI grade 2 flow, and eight patients had TIMI grade 3 flow. The results of the angiogram in patients who received Activase and placebo have not yet been reported (8).

Antman et al. also reported the results from the dose-finding and dose-confirmation phases of the TIMI-14 trial, which evaluated the use of thrombolytic therapy in combination with abciximab in patients with AMI (9).

TNKase—a new, genetically engineered variant of t-PA—is produced by recombinant DNA technology. TNKase is fibrin-specific. This fibrin specificity decreases systemic activation of plasminogen and the resulting breakdown of the circulating fibrinogen when compared to a molecule that lacks this feature. The ASSENT-2 trial was a phase III, randomized, double-blind trial that compared TNKase with Activase. Anticoagulants such as heparin and vitamin K antagonists, acetylsalicylic acid, dipyridamole, and GPIIb/IIIa inhibitors, may increase the risk of bleeding if administered prior to, during, or after TNKase therapy.

Combination strategies of LMWH with GPIIb/IIIa inhibitor or LMWH with a thrombolytic agent or thrombolytic agent with GPIIb/IIIa inhibitors may provide better approaches in the management of thrombosis or thromboembolic complications (10,11). Orally available drugs with rapid onset of action and no need for laboratory monitoring will be more suited for postsurgical prophylaxis of patients undergoing major hip or knee replacement surgeries than the LMWH or coumadin. A prodrug form of melagatran exhibits good bioavailability after oral administration, and has undergone phase II clinical evaluation for prophylaxis of thrombosis in orthopedic patients. For patients who develop venous thromboembolism (VTE) without identifiable risk factors requiring long-term oral anticoagulation, orally active drugs that target thrombin or factor Xa may
show better outcomes. With the dawning of the genomic era, future drug development and drug interactions that utilize microarray technology will go hand in hand with the diagnosis of disease or drug interactions at the genetic or molecular level.

Although the development of new antithrombotic and new anticoagulant drugs has been rather impressive, optimized use of aspirin, oral anticoagulants, and heparin has added a new dimension in the management of thrombotic disorders. Polytherapeutic approaches have been used to treat thrombotic disorders. The development of synthetic pentasaccharides represents a validation of the target specificity of heparins. Additional targets will be described in the near future, and heparins will provide other drugs with biochemical and functional specificities.

Pentasaccharide has undergone various phase II and phase III trials (12–15), and a recent publication has provided comparative evidence on the therapeutic effectiveness of this drug (16). However, it should be emphasized that pentasaccharide contains only one of the multiple pharmacological properties of heparin. Such a selective approach may have narrower therapeutic implications; however, in combination with other drugs this new antithrombotic oligosaccharide may provide similar effects to those observed with aspirin/clopidogrel combinations. The PENTATHLON study showed a rebound effect at 6.0 mg, and the rate of thrombosis was higher than in the lowered dose of 3.0 mg/kg. Furthermore, the bleeding was higher in the 3.0-mg dose in comparison to the comparative group in which enoxaparin was used. The rebound thrombotic effect is paradoxical, and may be explained on the basis of biochemical limitations. The fact that pentasaccharide produced an increased bleeding in the 3.0-mg group as compared to the 30-mg bid groups treated with enoxaparin was also an important consideration for the relatively higher rates of bleeding. An arbitrary dose of 2.5 mg was chosen for the additional studies. This is remarkable because there was no weight adjustment in these patients and with a statistical difference between 2.5 and 3.0 mg dose in a population with a weight of 60–90 kg, it is difficult to demonstrate the physical differences. In renal compromise, the accumulation of pentasaccharide can be readily attained, and one would expect a strong bleeding outcome. In the REMBRANDT trial, pentasaccharide demonstrated no significant differences between the dosages of 7.5 and 10 mg. This may be because of the saturation of AT by pentasaccharide. Interestingly, unlike the PENTATHLON study, in this study at a 10-mg dose, no bleeding complications were observed. If the hemorrhagic threshold of pentasaccharide is so low that a reduction of 0.5-mg dosage provides different results, then it may be more useful to adjust the dosage. Careful dosage selection of pentasaccharide is warranted before it is used in any combination therapy to avoid undesirable bleeding complications.
Drug Interactions

Oral anticoagulant drugs such as warfarin also exert their therapeutic effects by multiple mechanisms. These drugs are optimized for the management of thrombotic and cardiovascular disorders using the INR and the dosage optimization approaches. Oral heparin and oral antithrombin formulations are individually developed as potential replacements for the oral anticoagulant agents. The development of oral thrombin inhibitors and oral heparin formulations as a potential replacement for oral anticoagulants are rather significant. These drugs are currently in phase III clinical trials. However, marked variations in the oral absorption, metabolic conversion, alterations in hepatic function, and the absorption indices will markedly influence their bioavailability. It is difficult to predict whether these drugs will ever achieve therapeutic potential as oral anticoagulants. Warfarin has a track record in multiple indications, with an enormous clinical database. The apparent problems associated with warfarin may also be observed with oral heparin and thrombin inhibitors. These oral heparin and thrombin formulations have different degrees of bioavailabilities. Since a specific antagonist is not available, caution should be exercised—especially when these agents are used in combination with other anticoagulants to avoid uncontrolled bleeding complications. Careful calibration of dosages is warranted before any attempt to combine these oral formulations with other anticoagulant agents.

The current trend in the reperfusion therapy for AMI involves the use of fibrinolytic therapy in combination with more effective antithrombotic therapy to enhance early coronary patency and myocardial perfusion and to prevent reoclusion (17). The GUSTO V, ASSENT-3, and HERO-2 trials have shown that more effective antiplatelet and/or anticoagulant therapies can reduce myocardial infarction (MI) rates by more than 20% (18–20). Benefits of the adjunctive use of GPIIb/IIIa and coronary intervention have been demonstrated (21).

2. Combination Fibrinolytic and GPIIb/IIIa Inhibitor Therapy

Inhibition of the final common pathway of platelet aggregation is important, especially when these GPIIb/IIIa inhibitors are combined with fibrinolytic therapy. Initial results of Phase II angiographic studies, with half-dose t-PA (9), reteplase (22,23), or tenecteplase (24–26) when combined with either abciximab (9,23–25), eptifibatide (25,27), or tirofiban (26) markedly improved infarct artery TIMI grade 3 flow (9,22,27). These results were less consistent in confirmation phases (27), and were found to be less evident in later studies (23–25). Combination fibrinolytic and GPIIb/IIIa inhibitor showed greater extent of ST-segment resolution, as observed in most of the trials (9,24). This demonstrates the importance of platelet involvement in microvascular occlusion after fibrinolysis (28) and better early ST-segment resolution and improved
myocardial perfusion and survival because of combination fibrinolytic and more potent antiplatelet therapy (29).

The GUSTO V trial of 16,000 patients found no significant improvement in survival with half-dose reteplase and abciximab; however, the trial did demonstrate non-inferiority to reteplase alone (18). The secondary benefits of combination therapy included a lower rate of reinfarction (2.3% vs 3.5%) and less need for urgent coronary intervention. Although intracranial hemorrhages were the same at 0.6%, higher rates of ICH were observed in patients >75 yr of age. Thus, half-dose reteplase or tenecteplase combined with abciximab is an effective combination in young patients. A combination strategy of GPIIb/IIIa inhibitors with reduced-dose fibrinolytic therapy before acute angioplasty, or “facilitated angioplasty,” needs to be tested. A combination strategy of half-dose reteplase and abciximab followed by early angioplasty is safe, with good clinical outcomes (30). Randomized clinical trials involving the strategies of earliest combination pharmacologic reperfusion therapy combined with coronary intervention are now in progress.

3. Combination Fibrinolytic and LMWH Therapy

LMWHs in combination with fibrinolytic therapy have been evaluated in five randomized clinical trials (24,31–34).

1. The HART-2 study: The strategy of enoxaparin or unfractionated heparin (UFH) with t-PA was compared, and it was found that enoxaparin was at least as effective as UFH as an adjunct to t-PA, with higher recanalization rates and less occlusion at 5–7 d (31).
2. The ENTIRE study: This study compared enoxaprin to UFH, and full-dose to half-dose tenecteplase with abciximab in a factorial design (24). Enoxaparin showed similar TIMI 3 flow rates, and showed an advantage over UFH in regard to the ischemic events during a period of 30 d.
3. The ASSENT-PLUS study: This study compared dalteparin to UFH with t-PA (32).
4. The AMI-SK trial: This trial compared enoxaparin vs placebo with streptokinase, and demonstrated better coronary-artery patency at 8 d and reduced rates of infarction and recurrent ischemia with enoxaparin (33).
5. The ASSENT-3 trial: This trial evaluated the efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or UFH in 6000 patients. Enoxaparin significantly reduced, from 15.4 to 11.4%, the primary efficacy composite end points of death, reinfarction, and recurrent ischemia.

3.1. Ongoing Trials

1. The ASSENT-3 PLUS study: Approximately 1600 patients treated with tenecteplase are being randomized to enoxaparin vs UFH in the prehospital setting.
2. The EXTRACT trial: Approximately 21,000 patients will be evaluated to compare enoxaparin vs UFH with a fibrinolytic agent of the investigator’s choice to evaluate death and reinfarction in a more definitive manner.

4. Combination Fibrinolytic and Direct Thrombin Inhibitor Therapy

Over 36,000 patients with AMI treated with direct thrombin inhibitors in 11 randomized clinical trials showed a 15% relative reduction ($p = 0.001$) in the composite end points of death and MI at the end of treatment (35). The first AMI trial designed to evaluate mortality with a direct thrombin inhibitor—bivalirudin compared to heparin in patients treated with streptokinase—was the HERO-2 trial, which demonstrated no effect on mortality, but one-quarter reduction in reinfarction from 2.3% to 1.6% at 4 d ($p = 0.001$), with a minor increase in the risk of bleeding complications.

5. Experimental Evidence for the Interactions Between Antiplatelets and Anticoagulant Using Thrombelastography

5.1. Thrombelastography (TEG)

TEG has been used in various hospital settings since its development by Hartert in 1948 and others. The principle of TEG is based on the measurement of the physical viscoelastic characteristics of blood clots. Clot formation is monitored at 37°C in an oscillating plastic cylindrical cuvet ("cup") and a coaxially suspended stationary piston ("pin") with a 1-mm clearance between the surfaces. The cup oscillates in either direction every 4.5 s with a 1-s mid-cycle stationary period, resulting in a frequency of 0.1 Hz. The pin is suspended by a torsion wire that acts as a torque transducer. During clot formation, fibrin fibrils physically link the cup to the pin, and the rotation of the cup is transmitted to the pin via the viscoelasticity of the clot, is displayed on-line using an IBM-compatible personal computer and customized software (Haemoscope Corp., Skokie, IL). The torque experienced by the pin is plotted as a function of time, as shown by the different TEG clot parameters.

The following TEG parameters were monitored:

- **r**: The period of time of latency from the time that the blood was placed in the TEG® until the initial fibrin formation. R-time is prolonged by anticoagulants and is shortened by hypercoagulable states.

- **K**: K-time is a measure of the speed needed to reach a certain level (20 mm) of clot strength. K and $\alpha$ both measure similar information, and both are affected by the availability of fibrinogen, which determines the rate of clot buildup; in the presence of factor XIII, which enables crosslinking of fibrin to form a stable clot; and to a lesser extent, by platelets.
α: Measures the rapidity (kinetics) of fibrin buildup and crosslinking, that is the speed of clot strengthening. α is decreased by anticoagulants that affect fibrinogen and platelet function.

MA (maximum amplitude): A direct function of the maximum dynamic properties of fibrin and platelet bonding, which represents the ultimate strength of the platelet/fibrin clot. Maximum Amplitude (MA, in mm), is the peak rigidity manifested by the clot at 45–90 min.

5.2. Blood Sampling

Whole blood can be collected into siliconized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing 3.2% trisodium citrate, so that a ratio of citrate to whole blood of 1:9 (v/v) is maintained. TEG was performed within 3 h of blood collection on a slow-speed rocker.

Two different conditions can be used for blood collection and induction of clot formation. These include the following: i) Calcium was added back at an average of 2.25 mM concentration followed by the addition of tissue factor (TF) (25 ng/cup) for the in vitro studies. This CaCl₂ concentration showed only a minimal effect on clot formation and clot strength. ii) Recalcification by adding 10 mM calcium resulted in a similar peak MA. The in vitro effects of GPIIb/IIIa antagonists, anticoagulants such as heparin or LMWH, or a combination of both at sub-effective levels on platelet/fibrin clot dynamics were examined.

LMWHs are shown to act at multiple sites, including inhibition of factor Xa, inhibition of thrombin, and via the increase in cellular release of tissue-factor pathway inhibitor (TFPI). Platelet GPIIb/IIIa blockade represents the common pathway for platelet aggregation. The present study was undertaken to determine the interactions between the LMWH tinzaparin and various platelet GPIIb/IIIa antagonists, including abciximab, integrilin, tirofiban, or roxifiban, on TF-induced platelet fibrin-clot strength (PFCS). Computerized thrombelastography (TEG) was used to determine the ability of platelets and fibrin to augment human blood clot formation and strength under conditions of maximal platelet activation accelerated by TF in human blood. The effect of sub-effective concentrations of tinzaparin (20–30% PFCS inhibition) on the dose-response of the GPIIb/IIIa antagonists and vice versa was examined. Additionally, studies in dogs given sub-effective subcutaneous (sc) doses of roxifiban (0.1 mg/kg), tinzaparin (100 IU/kg), or combinations of both on ex vivo clot retraction induced by TF using TEG were determined. Under these conditions, platelets significantly enhance clot strength eightfold relative to platelet-free fibrin clots. Abciximab and roxifiban effectively inhibited this enhancement of clot strength. In contrast, integrilin or tirofiban were much less effective. The combination of sub-effective tinzaparin and roxifiban or abciximab resulted in distinct synergy in improving the antiplatelet and anticoagulant effect mediated...
Drug Interactions

Table 1
Effect of Tinzaparin and Integrilin on Inhibition of Platelet/Fibrin Clot

<table>
<thead>
<tr>
<th>Antithrombotics</th>
<th>Conc</th>
<th>% Inhibition of platelet/fibrin clot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinzaparin</td>
<td>0.1 µg/mL</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Tinzaparin</td>
<td>0.2 µg/mL</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>Integrilin</td>
<td>0.3 µM</td>
<td>0</td>
</tr>
<tr>
<td>Integrilin + tinzaparin</td>
<td>0.3 µM + 0.1 µg/mL</td>
<td>80 ± 9</td>
</tr>
<tr>
<td>Integrilin + tinzaparin</td>
<td>0.3 µM + 0.2 µg/mL</td>
<td>100 ± 0</td>
</tr>
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</table>

Data represent mean ± SD, n = 5.

by TF. Similar synergistic interactions were demonstrated after sc administration of roxifiban and tinzaparin at reduced doses in dogs. The in vitro dose-response relationship of PFCS using TF-TEG at reduced levels of tinzaparin with clinically achievable levels of tirofiban or integrilin significantly inhibited PFCS. These data suggest the potential of low-dose tinzaparin with either low-dose GPIIb/IIIa antagonists (abciximab or roxifiban) or with full-dose GPIIb/IIIa antagonists (integrilin or tirofiban) in the prevention and treatment of various thromboembolic disorders. The effect of tinzaparin on inhibition of platelet/fibrin clot are given in Table 1.

5.3. Need for Heparin

Results from PRISM, PRISM-PLUS, and PURSUIT suggest an important role for concomitant heparin in trials with intravenous (iv) GPIIb/IIIa inhibitors. Greater clinical benefit was shown in the patients who received the IIb/IIIa inhibitor plus heparin as compared to the IIb/IIIa inhibitor alone. These data suggested the potential benefit of heparin. Recent studies from our laboratory demonstrated a synergistic effect of heparin with class II GPIIb/IIIa antagonists in inhibiting platelet/fibrin clot dynamics. Additionally, heparin plus class I demonstrated additive effects in inhibiting clot dynamics.

Similar synergistic interactions were demonstrated between GPIIb/IIIa antagonists and thrombolytics, based on this in vitro model and based on experimental in vivo models of thrombosis as well as clinical investigations.

References


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