The direct thrombin inhibitor argatroban effectively prevents cardiac catheter thrombosis *in vitro*

Uwe Raaz; Anja Kaeberich; Lars Maegdefessel; Michael Buerke; Marese Busshardt; Sebastian Schubert; Martin Russ; Alexander Plehn; Henning Ebelt; Karl Werdan; Axel Schlitt

Department of Medicine III, Martin-Luther-University Halle-Wittenberg, Halle, Germany

Summary

The direct thrombin inhibitor argatroban offers some significant advantages over unfractionated heparin (UFH) and is recommended as an alternative anticoagulant during percutaneous coronary interventions (PCI). The impact of argatroban on cardiac catheter thrombosis – a severe potential complication of PCI – has not been systematically studied yet. The aim of the present study was to test *in vitro* the hypothesis that argatroban is equivalent to the more established anticoagulants UFH and enoxaparin in preventing catheter thrombus formation. Blood pretreated with the anticoagulants of interest was continuously circulated through a guiding catheter by using a roller pump for a maximum experimental period of 60 minutes. In an alternate model, coagulation was mechanically induced by a magnetic stirrer. Coagulation parameters, overall thrombus weight and electron microscopic features (deposits of platelets and fibrin on the catheter surface) were quantified as endpoints. Argatroban (administered as bolus or continuous infusion), UFH (bolus), and enoxaparin (bolus) significantly reduced catheter thrombus formation compared to untreated controls. Here, neither overall thrombus weight nor platelet/fibrin deposition was different among the specific anticoagulants. Declining ACT (activated clotting time) levels – which were found in the argatroban bolus group – could be prevented by continuous infusion. In magnetic stirrer-induced coagulation, thrombus weight was lower following bolus treatment with UFH and enoxaparin compared to argatroban. These data suggest that the potential for argatroban in preventing catheter thrombosis is comparable to that of UFH and enoxaparin. However, the anticoagulatory efficacy varied, depending on the model of coagulation activation, which demonstrates the necessity for specific testing.

Keywords

Anticoagulation, direct thrombin inhibitors, PCI

Correspondence to:
Uwe Raaz, MD
Department of Medicine III, Martin-Luther-University
Ernst-Grube-Str. 40, 06097 Halle, Germany
Tel.: +49 345 557 7433, Fax: +49 345 557 4951
E-mail: uwe.raaz@medizin.uni-halle.de

Introduction

Systemic anticoagulants represent a cornerstone in the contemporary medical management of acute coronary syndromes (ACS) (1–3). However, unfractionated heparin (UFH) – which since its discovery in 1916 remains the most frequently employed anticoagulant in ACS – exhibits important drawbacks, such as platelet activation, rebound phenomena, or immunogenic complications [e.g. heparin-induced thrombocytopenia (HIT)], which have stimulated the development of more effective and safer antithrombin agents (4, 5).

Direct thrombin inhibitors (DTI) constitute a recently developed class of antithrombin agents which target the thrombin molecule as a central factor of the coagulation cascade (6). In contrast to UFH, DTI inhibit thrombin *directly* – i.e. without the presence of antithrombin being indispensable – and regardless of whether thrombin is soluble or bound to fibrin. DTI do not stimulate platelet activation and aggregation, which may be regarded as a crucial advantage over heparins, especially in the context of ACS (4). Furthermore, due to a low immunogenic potential and their structural difference from heparins, DTI represent a valuable alternative to heparins in the context of HIT (7). Consequently, contemporary guidelines recommend the DTI bivalirudin or argatroban as alternatives to heparin during percutaneous coronary interventions (PCI), especially with respect to HIT (8, 9).

Argatroban, a synthetic DTI derived from L-arginine, reversibly binds the active site of thrombin and prevents fibrin formation and platelet aggregation without having an inhibitory effect on other serine proteases at therapeutic concentrations (10). Indeed, argatroban exhibits predictable pharmacodynamic responses, as measured by either the activated partial thromboplastin time (aPTT) or activated clotting time (ACT), and is therefore increasingly being employed in PCI for ACS (11–13). In contrast to ximelagatran, an orally available DTI which was withdrawn from the

market due to an increased risk of liver toxicity after prolonged administration, argatroban seems to be safe and moreover – after proper dose adjustment – may provide safe and effective antithrombotic therapy in patients with hepatic impairment (14, 15).

With respect to PCI, clinical investigation of modern anticoagulants in ACS has revealed another important aspect of antithrombin therapy, namely, the prevention of thrombus formation on the cardiac catheter equipment (i.e. catheter thrombosis). For example, the OASIS-5 and OASIS-6 trials revealed an increased incidence of catheter thrombosis in patients with ACS who received the selective factor Xa inhibitor fondaparinux for systemic anticoagulation (16, 17).

As catheter thrombus formation can result in disastrous consequences during PCI, our group has focused on this particular issue of anticoagulation using an *in vitro* model. Given the therapeutic significance of PCI, all anticoagulants employed or tested in the setting of ACS should also effectively prevent catheter thrombosis formation.

The aim of the present study was to test *in vitro* the hypothesis that argatroban is equivalent to the more established anticoagulants UFH and enoxaparin in preventing catheter thrombus formation.

Materials and methods

Blood sample preparation and experimental processing (cardiac catheter)

Blood (50 ml) from 10 healthy male donors was used for each anticoagulatory condition investigated, whereby donors served as their own control. All blood donors took 500 mg aspirin orally 2 hours (h) before blood was drawn.

The blood was venesected directly into a 50 ml tube which was prepared with the specific anticoagulants of interest, namely: 1) no anticoagulant (control); 2) argatroban – bolus 3 µg/ml + eptifibatide 1.7 µg/ml; 3) argatroban – infusion (3 µg/ml + 2.5 µg/ml/h); 4) argatroban – infusion + eptifibatide; 5) enoxaparin 0.6 U/ml; 6) enoxaparin + eptifibatide; 7) UFH 0.8 U/ml; and 8) UFH plus eptifibatide. The concentrations of enoxaparin and UFH were adopted from previous studies (18, 19) as they were found effective to induce levels of anti-Xa activity (enoxaparin) and ACT (UFH) as recommended for PCI (9, 20). The concentration of argatroban was determined empirically to reach ACT levels appropriate for PCI (7, 9, 21). Subsequently, experiments were performed as described previously (18). Briefly, blood from the tube (preserved in a 37°C water bath) was circulated via a 6F multipurpose guiding catheter (Cordis®) by a roller pump (Masterflex®) at a flow rate of approximately 3 ml/minute (min) for a maximum experimental duration of 60 min. If the catheter was occluded before 60 min of circulation had elapsed, this time was recorded. Additional experiments without catheter perfusion were performed.

Blood preparation and experimental processing (magnetic stirrer)

For further characterisation the anticoagulants were tested in an alternative model of mechanically induced coagulation activation.

Similarly to the protocol described for the cardiac catheter, blood (45 ml) was venesected directly into a 60 ml specimen bar prepared with the anticoagulants as follows: 1) UFH (0.8 U/ml); 2) argatroban (3 µg/ml); and 3) enoxaparin (0.6 U/ml). Subsequently, blood was stirred by a magnetic stirrer for 60 min at 37°C. Afterwards the thrombus adherent to the stirrer was analysed qualitatively (photograph) and quantitatively (stirrer weight).

The study was approved by the ethics committee of the Martin-Luther-University, Halle-Wittenberg, Germany. The blood donors were volunteers and all participants gave their written, informed consent.

Thrombus weight quantification

To quantify the weight of the thrombi, each catheter was weighed before and after the individual experiments (catheter model). The magnetic stirrer (identical for all experiments) was weighed after the experimental protocol was completed (stirrer model).

Electron microscopy studies

For a more detailed analysis of thrombus formation, the degree of platelets and fibrin deposits on the catheter surface was determined by electron microscopy. For that purpose catheters were prepared as described previously (18). Briefly, catheters were cleansed of blood using Soerensen solution that was introduced into the circulation for approximately 60 seconds (sec). Catheter-adherent thrombi were then fixed by glutaraldehyde. Subsequently, catheter tips (1 cm) were cut longitudinally and then dehydrated in a graded series of acetone solution, treated with hexamethyldisilazane to prevent oxidation, and dried overnight under an exhaust system.

For the scanning electron microscopic analysis, a LEO 1530 scanning electron microscope (Gatech®, Georgia Institutes of Technology, Atlanta, GA, USA) was used. A 100x150 µm region central to the catheter tips was photographed and, subsequently, the number of platelets in this area was counted. Fibrin deposition was quantified according to a scale from 0 – 4 (0= no fibrin deposition, 1= trace fibrin deposition, 2= low fibrin deposition, 3= moderate fibrin deposition, and 4= heavy fibrin deposition). All microscopic analyses were undertaken by two independent investigators blinded to the anticoagulant used.

Determination of coagulation parameters (ACT, anti-Xa activity, T-AT)

To determine and compare the respective anticoagulation levels ACT was quantified by using a Medtronic ACT PLUS® device and anti-Xa activity was detected by using the routine laboratory methods employed at Martin Luther-University Halle-Wittenberg, Germany. T-AT was quantified as a measure of thrombin generation using the ENZYGNOST TAT micro ELISA (Dade Behring, Marburg, Germany).

Statistics

One-way ANOVA with post-hoc analysis was performed for comparisons between experimental groups. P-values <0.05 were considered to be statistically significant.

Results

Catheter thrombus weight and catheter circulation time

Thrombus weight was taken as the overall quantitative marker of thrombus formation under the different anticoagulants investigated. Additionally, catheter circulation time was recorded as a gross indicator of thrombus formation that leads to complete catheter occlusion.

Blood that had not been treated with an anticoagulant (serving as control) completely occluded the catheter after an average of 5.8 ± 1.1 min, whereas for all anticoagulants tested the catheter remained patent for the entire or nearly entire experimental time of 60 min (argatroban – bolus + eptifibatide: 54.7 ± 12.6 min; argatroban – infusion: 59.2 ± 1.9 min; argatroban – infusion + eptifibatide: 59.8 ± 0.6 min; UFH: 60 ± 0 min; UFH + eptifibatide: 60 ± 0 min; enoxaparin: 59.4 ± 2 min; enoxaparin + eptifibatide: 60 ± 0 min).

Thrombus weight was significantly lower in all anticoagulant groups than in untreated controls (Fig. 1). However, no statistically significant difference was observed among the specific anticoagulants tested (Fig. 1). Additional treatment with eptifibatide did not affect thrombus weight regardless of whether it was administered in combination with argatroban, UFH, or enoxaparin (Fig. 1).

Parameters of anticoagulation

Anti-Xa activity – which was assessed to monitor low-molecular-weight heparin-induced anticoagulation – was not significantly different between enoxaparin (0.77 ± 0.18 IU/ml) and enoxaparin + eptifibatide (0.81 ± 0.06 IU/ml) regimen.

ACT was used as a parameter of anticoagulation achieved by argatroban and UFH treatment. Here, we found no difference between the individual treatment strategies using argatroban or UFH
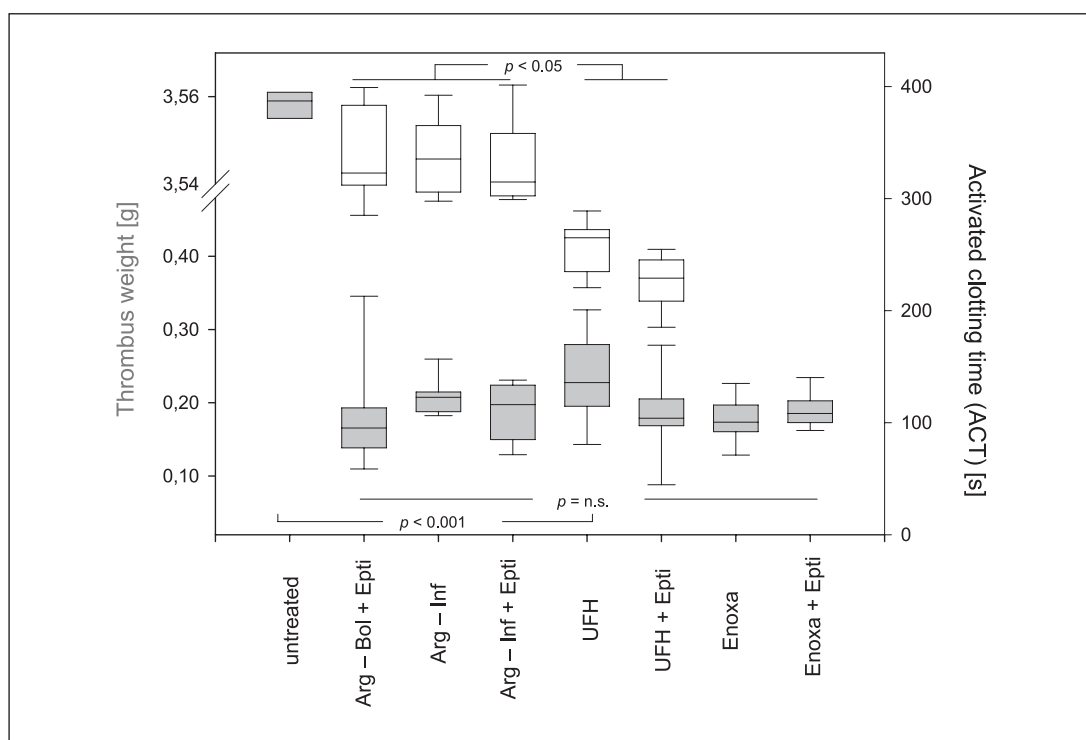
Figure 1: The influence of argatroban, UFH, and enoxaparin on catheter thrombus weight and activated clotting time. Box plots depict thrombus weight (gray) and ACT (white; except for control and enoxaparin) under different conditions of anticoagulation. Arg – Bol indicates argatroban bolus administration; Arg – Inf, argatroban continuous infusion; enoxa, enoxaparin; UFH, unfractionated heparin; epti, eptifibatide. Values are mean ± SD.

(Fig. 1). However, ACT was lower in the UFH groups than for the argatroban regimen (Fig. 1).

Comparison of the ACT time course between the argatroban bolus and argatroban infusion regimen showed that ACT was significantly lower in the bolus group than under infusion conditions, where ACT levels remained constant (Fig. 2). To investigate presumed thrombin-related argatroban consumption as an explanation for these declining ACT levels, thrombin-antithrombin complexes (T-AT) were quantified as a measure of thrombin generation. Here we found plasmatic T-AT levels significantly increasing during the experimental course (Fig. 3).

Electron microscopy studies

Electron microscopy was performed to evaluate catheter-associated thrombus formation in more detail.

Fibrin deposition on the catheter tips did not significantly differ for any of the specific anticoagulant treatments tested (argatroban – bolus + eptifibatide: 1.1 ± 0.6; argatroban – infusion: 2.2 ± 1.5; argatroban – infusion + eptifibatide: 1.1 ± 0.6; UFH: 1.0 ± 0.6; UFH + eptifibatide: 1.0 ± 0.2; enoxaparin: 2.2 ± 1.6; enoxaparin + eptifibatide: 1.8 ± 1.0). At the same time, the number of platelets adherent to the catheter was similar (i.e. not significantly different; argatroban – bolus + eptifibatide: 389 ± 110; argatroban – infusion: 226 ± 203; argatroban – infusion + eptifibatide: 211 ± 162; UFH: 339 ± 225; UFH + eptifibatide: 201 ± 116; enoxaparin: 121 ± 86; enoxaparin + eptifibatide: 341 ± 267.

Magnetic stirrer

The magnetic stirrer was employed as an alternative model of coagulation activation under mechanically challenging conditions.

Investigating the different anticoagulants following bolus administration we qualitatively (photographic thrombus aspect; Fig. 4B) and quantitatively (stirrer weight; Fig. 4A) found that thrombus generation was lower under UFH and enoxaparin treatment compared to argatroban.

Discussion

DTIs are emerging as a promising class of anticoagulants in the management of acute coronary syndromes. Data from the RE-PLACE-2 trial and the ACUITY trial have established that bivalirudin, a member of the DTI family, is a safe and effective agent that – compared to heparins – reduces major bleedings without increasing thrombotic events in both lower- and higher-risk patients undergoing PCI (22, 23).

Argatroban, a synthetic DTI with a distinct pharmacologic profile different from that of bivalirudin (24, 25) – lower affinity to
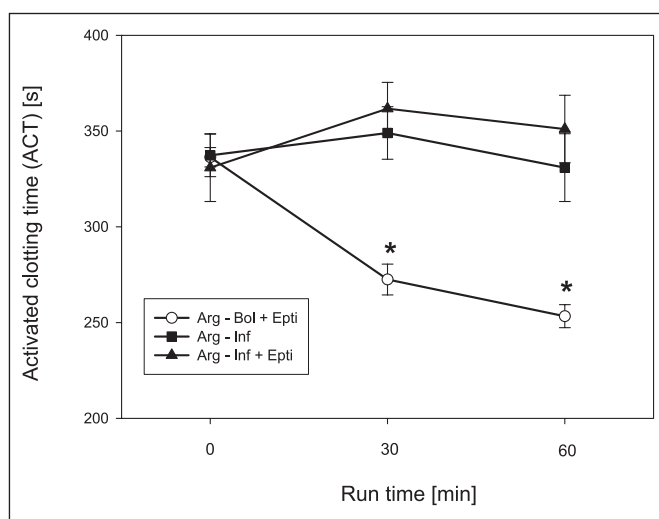
Figure 2: Course of activated clotting time (ACT) during catheter thrombosis experiments under different modes of argatroban administration. Arg – Bol indicates argatroban bolus administration; Arg – Inf, argatroban continuous infusion; epti, eptifibatide. * indicates $p < 0.05$ vs. control ACT at t=0 and vs. corresponding ACTs at t=30 and t=60, respectively. Values are mean ± SEM.
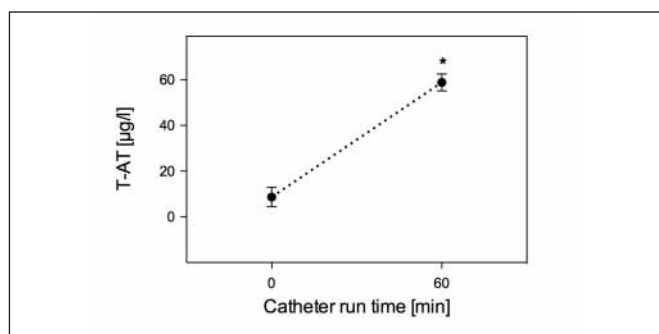
Figure 3: T-AT plasma levels following argatroban bolus administration (Arg – Bol) before and after catheter circulation. * indicates $p < 0.05$ vs. control T-AT at t=0. Values are mean ± SD.

thrombin (Ki=40 nM vs. Ki=2 nM), different mode of elimination (hepatobiliary excretion vs. non-organ-dependent proteolysis) – is another agent increasingly being employed during PCI, especially in patients with a history of or with acute HIT (11, 26).

Cardiac catheter thrombosis is a rare but potentially disastrous complication in the context of PCI (27). As this aspect of anticoagulation is highly controversial – reports from recent clinical studies range from sufficient antiplatelet-only regimen without systemic anticoagulation for simple elective PCI to a superiority of UFH over enoxaparin (28, 29) – our group sought to focus in particular on catheter thrombosis using an *in vitro* approach. Having developed a sensitive model of cardiac catheter thrombosis we were able to reproduce, at the *in vitro* level, the higher incidence of catheter thrombosis following anticoagulation with fondaparinux initially reported from clinical trials (18).

© Schattauer 2010
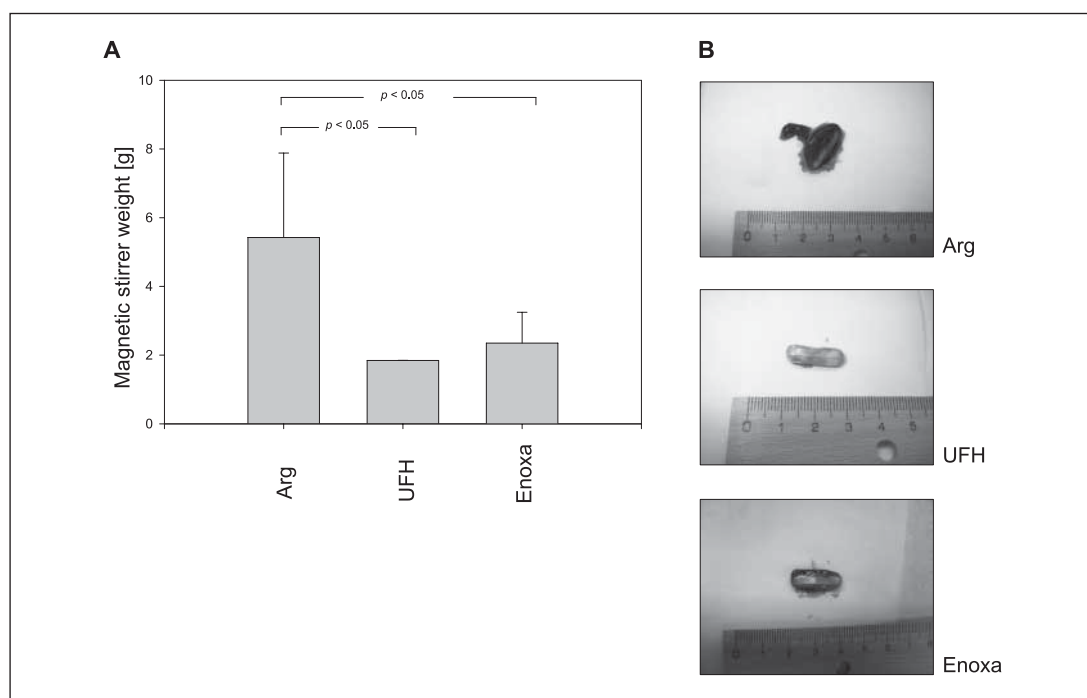
Figure 4: The influence of argatroban, UFH, and enoxaparin on magnetic stirrer thrombus generation. Bars depict overall stirrer weight (A), photographs show a corresponding visual aspect of thrombus generation (B). Arg indicates argatroban; UFH, unfractionated heparin, enoxa, enoxaparin. Values are mean ± SD.

As clinical data concerning argatroban usage in PCI as compared to bivalirudin are limited, the present study was designed to test the hypothesis that argatroban is equivalent to UFH and enoxaparin in preventing catheter thrombus formation.

Here, we found that all anticoagulants tested (argatroban, UFH, and enoxaparin) significantly reduced catheter thrombus formation in comparison to controls without anticoagulant treatment – once again demonstrating the necessity of employing anticoagulants to counteract catheter surface thrombogenicity. Although we observed some slight differences concerning total circulation time, our catheter model demonstrated a comparable efficacy of all anticoagulatory conditions tested with regard to overall thrombus weight and microscopic features of thrombus formation, such as catheter-adherent platelets or fibrin deposits.

Of note is that the initial ACTs – which all reached levels appropriate for PCI – were higher in the groups employing argatroban than under UFH-treated conditions (Fig. 1). However, it is known that ACTs obtained by the Medtronic ACT PLUS® device (Hemo-Tec system) – which was used in this study – are relatively higher under argatroban medication and lower under heparin treatment as compared to other monitoring devices (30). In this respect target ACT level recommendations for PCI are different for UFH (250–300 sec by HemoTec) and argatroban (300–450 sec, regardless of the device) (7, 9, 21). Furthermore, under argatroban given as a bolus medication the declining ACT levels – as quantified after 30 and 60 min (Fig. 2) – still prevented the formation of thrombi in the catheter (Fig. 1).

In contrast to our previous data investigating the effects of bivalirudin on cardiac catheter thrombosis, argatroban inhibited thrombus formation regardless of whether it was administered as a bolus or continuously (19). This finding might be explained by the different modes of DTI elimination: bivalirudin is cleaved by organ-independent proteolysis, whereas argatroban is excreted via the hepatobiliary system, which obviously is not present in an *in vitro* setting (24). Nevertheless, our temporal ACT analyses (Fig. 2) revealed a decline in an argatroban-related effect, suggesting *in vitro* drug elimination. This phenomenon might be explained on a

What is known about this topic?

- In interventional cardiology sufficient anticoagulation is critical for the prevention of cardiac catheter thrombosis as a potentially severe complication during percutaneous coronary interventions (PCI).
- The direct thrombin inhibitor argatroban is recommended as alternative to heparin during PCI, especially in patients with heparin-induced thrombocytopenia (HIT).
- In the light of a variable potential among different types of anticoagulants to prevent cardiac catheter thrombosis the anticoagulatory profile of argatroban has not been systematically evaluated yet.

What does this paper add?

- In our *in vitro* study argatroban (administered as bolus or continuous infusion) significantly reduces catheter thrombus formation equivalently to unfractionated heparin and enoxaparin.
- However, argatroban also exhibits a variable anticoagulatory efficacy depending on the mechanism of coagulation activation.
- In conclusion the data of this study encourage the use of argatroban for anticoagulation during PCI but also demonstrate a necessity for specific testing for specific anticoagulatory demands.

pharmacodynamic level by functional consumption of argatroban due to increasing thrombin generation during catheter circulation (Fig. 3): Under conditions of argatroban bolus administration a limited amount of argatroban is progressively occupied by a steadily increasing amount of thrombin that in turn results in a decreasing "thrombin inhibition capacity" as reflected by declining ACTs. Progressive thrombin generation is likely to result from continuous "contact activation" during catheter perfusion (as described below).

At first glance our present findings contradict previous data which found argatroban bolus administration to be inferior to continuous infusion in preventing thrombus formation on mechanical heart valves *in vitro* (31).

To focus on this discrepancy we additionally employed an alternate model of mechanical coagulation activation using a magnetic stirrer. In this experimental setup argatroban was inferior to UFH and enoxaparin in preventing appositional thrombus growth. Those findings might perhaps be explained by the qualitative and quantitative differences concerning the activation of the coagulation cascade. On artificial surfaces, thrombogenicity seems primarily to be a function of *local* thrombin generation rather than of platelet adhesion (32).

In a process termed "contact activation" coagulation factor XII (FXII; Hageman factor) undergoes autoactivation (FXIIa) following blood exposure to artificial surfaces (33). FXIIa then activates FXI thereby initiating a waterfall cascade that finally results in thrombin generation. Furthermore FXIIa is capable of activating the kallikrein-kinin system – which may independently induce thrombin formation (34).

However, in a setting of mechanical (shear) stress-induced coagulation, a pivotal role has been assigned to platelet activation/mechanical platelet destruction – which, in turn, potently activates and propagates the plasmatic coagulation cascade (35–37). Here, the extent of mechanical stress also determines the extent of platelet activation (37). Thus, in contrast to the low-flow conditions in our cardiac catheter model – where *surface activation* may be the critical event – in a scenario of mechanical heart valves as well as in our stirrer model the extent of coagulation might be dominated more by *mechanical force activation* that induces the coagulation cascade more extensively. This, in turn, might overload the anticoagulatory potential of DTI such as argatroban under conditions of "single shot" administration and might favour anticoagulants such as UFH and enoxaparin which inhibit the coagulation cascade at multiple sites.

Taken together, the results of this *in vitro* study show a significant potential of argatroban in preventing catheter thrombus formation. From that point of view, one could encourage the use of argatroban for anticoagulation during PCI. However, this study also reflects a differing efficacy of anticoagulants under varying conditions (heart valves, cardiac catheters), which demonstrates that it may not be adequate to simply extrapolate data from one study to another scenario.

Limitations

In contrast to current clinical practice, where most elective PCI procedures are performed in patients receiving established dual anti-platelet therapy (aspirin, clopidogrel), our blood donors were pretreated with aspirin only. Our study scenario, therefore, may be more analogous to PCI in the setting of ACS when active metabolites of clopidogrel are not present.

Acknowledgements

Abbreviations

ACS, acute coronary syndromes; ACT, activated clotting time; DTI, direct thrombin inhibitor; PCI, percutaneous coronary intervention; T-AT, thrombin-antithrombin complexes; UFH, unfractionated heparin.

References

1. Bassand JP, Hamm CW, Ardissino D, et al. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. Eur Heart J 2007; 28: 1598–1660.

2. Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction): developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons: endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. Circulation 2007; 116: e148-e304.

3. Antman EM, Hand M, Armstrong PW, et al. 2007 Focused Update of the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: developed in collaboration With the Canadian Cardiovascular Society endorsed by the American Academy of Family Physicians: 2007 Writing Group to Review New Evidence and Update the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction, Writing on Behalf of the 2004 Writing Committee. Circulation 2008; 117: 296–329.

4. Xiao Z, Theroux P. Platelet activation with unfractionated heparin at therapeutic concentrations and comparisons with a low-molecular-weight heparin and with a direct thrombin inhibitor. Circulation 1998; 97: 251–256.

5. Jang IK, Hursting MJ. When heparins promote thrombosis: review of heparin-induced thrombocytopenia. Circulation 2005; 111: 2671–2683.

6. Lepor NE. Anticoagulation for acute coronary syndromes: from heparin to direct thrombin inhibitors. Rev Cardiovasc Med 2007; 8 (Suppl 3): S9–17.

7. Lewis BE, Hursting MJ. Direct thrombin inhibition during percutaneous coronary intervention in patients with heparin-induced thrombocytopenia. Expert Rev Cardiovasc Ther 2007; 5: 57–68.

8. Silber S, Albertsson P, Aviles FF, et al. Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. Eur Heart J 2005; 26: 804–847.

9. Smith SC, Jr., Feldman TE, Hirshfeld JW, Jr., et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update the 2001 Guidelines for Percutaneous Coronary Intervention). J Am Coll Cardiol 2006; 47: e1–121.

10. Hursting MJ, Alford KL, Becker JC, et al. Novastan (brand of argatroban): a small-molecule, direct thrombin inhibitor. Semin Thromb Hemost 1997; 23: 503–516.

11. Cruz-Gonzalez I, Sanchez-Ledesma M, Baron SJ, et al. Efficacy and safety of argatroban with or without glycoprotein IIb/IIIa inhibitor in patients with heparin induced thrombocytopenia undergoing percutaneous coronary intervention for acute coronary syndrome. J Thromb Thrombolysis 2008; 25: 214–218.

12. Hirahara T, Kubo N, Ohmura N, et al. Prospective randomized study of argatroban versus heparin anticoagulation therapy after percutaneous coronary intervention for acute myocardial infarction. J Cardiol 2004; 44: 47–52.

13. Jang IK, Lewis BE, Matthai WH, Jr., et al. Argatroban anticoagulation in conjunction with glycoprotein IIb/IIIa inhibition in patients undergoing percutaneous coronary intervention: an open-label, nonrandomized pilot study. J Thromb Thrombolysis 2004; 18: 31–37.

14. Agnelli G, Eriksson BI, Cohen AT, et al. Safety assessment of new antithrombotic agents: lessons from the EXTEND study on ximelagatran. Thromb Res 2009; 123: 488–497.

15. Levine RL, Hursting MJ, McCollum D. Argatroban therapy in heparin-induced thrombocytopenia with hepatic dysfunction. Chest 2006; 129: 1167–1175.

16. Yusuf S, Mehta SR, Chrolavicius S, et al. Comparison of fondaparinux and enoxaparin in acute coronary syndromes. N Engl J Med 2006; 354: 1464–1476.

17. Yusuf S, Mehta SR, Chrolavicius S, et al. Effects of fondaparinux on mortality and reinfarction in patients with acute ST-segment elevation myocardial infarction: the OASIS-6 randomized trial. J Am Med Assoc 2006; 295: 1519–1530.

18. Schlitt A, Rupprecht HJ, Reindl I, et al. In-vitro comparison of fondaparinux, unfractionated heparin, and enoxaparin in preventing cardiac catheter-associated thrombus. Coron Artery Dis 2008; 19: 279–284.

19. Maegdefessel L, Buerke M, Schubert S, et al. Comparison of bivalirudin, enoxaparin, and unfractionated heparin in preventing cardiac catheter thrombosis. Results of an in-vitro study. Thromb Haemost 2008; 100: 693–698.

20. Montalescot G, White HD, Gallo R, et al. Enoxaparin versus unfractionated heparin in elective percutaneous coronary intervention. N Engl J Med 2006; 355: 1006–1017.

21. Iqbal O, Ahmad S, Lewis BE, et al. Monitoring of argatroban in ARG310 study: potential recommendations for its use in interventional cardiology. Clin Appl Thromb Hemost 2002; 8: 217–224.

22. Lincoff AM, Bittl JA, Harrington RA, et al. Bivalirudin and provisional glycoprotein IIb/IIIa blockade compared with heparin and planned glycoprotein IIb/IIIa blockade during percutaneous coronary intervention: REPLACE-2 randomized trial. J Am Med Assoc 2003; 289: 853–863.

23. Stone GW, McLaurin BT, Cox DA, et al. Bivalirudin for patients with acute coronary syndromes. N Engl J Med 2006; 355: 2203–2216.

24. Warkentin TE, Greinacher A, Koster A. Bivalirudin. Thromb Haemost 2008; 99: 830–839.

25. Swan SK, Hursting MJ. The pharmacokinetics and pharmacodynamics of argatroban: effects of age, gender, and hepatic or renal dysfunction. Pharmacotherapy 2000; 20: 318–329.

26. Hirahara T, Kubo N, Ohmura N, et al. Prospective randomized study of argatroban versus heparin anticoagulation therapy after percutaneous coronary intervention for acute myocardial infarction. J Cardiol 2004; 44: 47–52.

27. Grayburn PA, Willard JE, Brickner ME, et al. In vivo thrombus formation on a guidewire during intravascular ultrasound imaging: evidence for inadequate heparinization. Cathet Cardiovasc Diagn 1991; 23: 141–143.

28. Stabile E, Nammas W, Salemme L, et al. The CIAO (Coronary Interventions Antiplatelet-based Only) Study: a randomized study comparing standard anticoagulation regimen to absence of anticoagulation for elective percutaneous coronary intervention. J Am Coll Cardiol 2008; 52: 1293–1298.

29. Dana A, Nguyen CM, Cloutier S, et al. Macroscopic thrombus formation on angioplasty equipment following antithrombin therapy with enoxaparin. Catheter Cardiovasc Interv 2007; 70: 847–853.

30. Chia S, Van Cott EM, Raffel OC, et al. Comparison of activated clotting times obtained using Hemochron and Medtronic analysers in patients receiving antithrombin therapy during cardiac catheterisation. Thromb Haemost 2009; 101: 535–540.

31. Maegdefessel L, Linde T, Michel T, et al. Argatroban and bivalirudin compared to unfractionated heparin in preventing thrombus formation on mechanical heart valves. Results of an in-vitro study. Thromb Haemost 2009; 101: 1163–1169.

32. Keuren JF, Wielders SJ, Willems GM, et al. Fibrinogen adsorption, platelet adhesion and thrombin generation at heparinized surfaces exposed to flowing blood. Thromb Haemost 2002; 87: 742–747.

33. Schmaier AH. The elusive physiologic role of Factor XII. J Clin Invest 2008; 118: 3006–3009.

34. Stief TW. Kallikrein activates prothrombin. Clin Appl Thromb Hemost 2008; 14: 97–98.

35. Kroll MH, Hellums JD, McIntire LV, et al. Platelets and shear stress. Blood 1996; 88: 1525–1541.

36. Miyazaki Y, Nomura S, Miyake T, et al. High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. Blood 1996; 88: 3456–3464.

37. Williams MS, Coller BS, Vaananen HJ, et al. Activation of platelets in platelet-rich plasma by rotablation is speed-dependent and can be inhibited by abciximab (c7E3 Fab; ReoPro). Circulation 1998; 98: 742–748.