

Editorial Focus

Do we need thrombin generation assays for monitoring anticoagulation?

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Protamine sulphate neutralizes the anticoagulant effect of unfractionated heparin (UFH), and this effect can easily be demonstrated by a variety of assay systems, including the traditional activated partial thromboplastin time (aPTT), as well as anti-factor Xa assays, or thrombin generation tests such as the Calibrated Automated Thrombogram (CAT) (1). Protamine also neutralizes the anticoagulant effect of low-molecular-weight heparins (LMWH), but to a lesser and variable extent (2). In this issue of *Thrombosis and Haemostasis* Gatt et al. (3) compared the effect of protamin sulphate and other drugs on the effect of UFH, LMWH, danaparoid and fondaparinux on aPTT, anti-factor Xa-level, and a thrombin generation test. Reversal of anticoagulation is an interesting model for testing the performance of monitoring assays for anticoagulant drugs.

One important finding of the present paper by Gatt et al. is that protamine has little effect on apparent anti-factor Xa-activity levels of LMWH, but a considerable effect on the CAT results. This suggests that the success of protamine administration cannot be monitored by measuring anti-factor Xa activity. This is in some contrast to other investigations showing that anti-factor Xa activity of LMWH is affected by protamine (4, 5). Finally, protamine has no effect on the anticoagulant effect of danaparoid and fondaparinux, and this was evident in both anti-factor Xa-activity and CAT assays.

Reversal of danaparoid and fondaparinux, and the conventional LMWH is seldom necessary, but these drugs display a considerably prolonged half-life in patients with impaired renal function and severe bleeding may occur if the dose is not adjusted appropriately (6). Monitoring of the anticoagulant effect in patients with impaired renal function and in critically ill patients in general is essential for prevention of bleeding complications, and an assay system that gives a more global image of haemostatic function than an anti-factor Xa assay might be advantageous (7).

Recombinant factor VIIa (rFVIIa, NovoSeven®) has been suggested for treatment of patients with severe anticoagulant-induced bleeding (8), and several case reports have been published on the use of rFVIIa in bleeding fondaparinux-treated patients

(9, 10). As shown by Gatt et al. (3), the anti-factor Xa levels are not influenced by rFVIIa, whereas the CAT results reflect the procoagulant effect of rFVIIa. FEIBA® is an alternative to rFVIIa in the treatment of haemophilia patients with inhibitors, and may also be helpful in anticoagulant-induced bleeding. In the present experiments of Gatt et al., FEIBA acted quite similar to rFVIIa, although rFVIIa seemed to be more efficient.

Another interesting result is that fresh frozen plasma reduces the anti-factor Xa-activity of UFH, LMWH, danaparoid, and fondaparinux, but has little effect on the anticoagulant effect measured by CAT. The main effect of fresh frozen plasma is dilution, and it might be that the threshold concentration of the anticoagulants for an effect on the assay systems is different. Additional experiments might be needed to determine the threshold concentration of LMWH, danaparoid and fondaparinux for the CAT. From the present results, and experiments by other investigators it seems that thrombin generation tests more reliably reflect the anticoagulant effect of these drugs (11).

Anticoagulant drugs delay clot formation and traditional methods for the monitoring of anticoagulation are based precisely on this effect. After addition of an activator, clot formation occurs when only about 5% of the prothrombin has been activated to form thrombin. There are many theories why additional thrombin is formed after clot formation. The additional thrombin may not only improve clot stability by activating factor XIII and TAFI, induce cellular invasion or activation of endothelial cells adjacent to the clot *in vivo*, but also serves to induce the anticoagulant protein C-dependent feedback control of thrombin generation. Thrombin generation assays not only detect delayed thrombin generation (leading to a prolonged clotting time), but also reduced thrombin activity (leading to changes in structural and functional properties of the clot). Both may be relevant for the therapeutic effect of anticoagulant drugs.

The results of Gatt et al. (3) indicate that the CAT may be a prototype for global coagulation assays used for monitoring of anticoagulant therapy. New anticoagulants are entering the scene and one of the major advantages of these drugs is the reliable anticoagulant effect with little intra- and inter-individual varia-

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bility. Monitoring of the anticoagulant effect, which is indispensable in vitamin K-antagonist treatment, is only necessary at few occasions. In case of bleeding, it is helpful to know if the reason is overdose. Impaired renal or hepatic function may have an influence on pharmacokinetics as well. Before surgery, having an estimate of residual anticoagulant activity of the drugs may prevent unnecessary bleeding complications. Finally, if reversal of the anticoagulant effect becomes necessary in case of severe bleeding or urgent surgery, the influence of the reversing agents needs to be closely monitored.

An assay system for anticoagulation monitoring should accurately reflect the effect of any anticoagulant therapy, including sum effects of combinations of anticoagulants, and possibly also the effect of combinations of anticoagulants and platelet func-

tion inhibitors. The CAT may be such an assay, but additional data are needed to prove this. Also, it remains to be established, which parameter of the CAT is most relevant concerning the anticoagulant effect, as the CAT appears to underestimate the contribution of factor Xa inhibition (12, 13). Factor Xa inhibitors delay thrombin generation and reduce the total amount of thrombin formed (14), whereas direct thrombin inhibitors mainly act as scavengers of active thrombin, thereby delaying the thrombin burst without reducing the final amount of thrombin formed.

In the future thrombin generation tests may replace the traditional assays for monitoring of anticoagulant drugs. Still, substance-specific assays will be needed in addition to the sum function provided by the thrombin generation tests, for monitoring of transitions from one anticoagulant to another.

References

1. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003; 33: 4–15.
2. Makris M, Hough RE, Kitchen S. Poor reversal of low molecular weight heparin by protamine. *Br J Haematol* 2000; 108: 884–885.
3. Gatt A, van Veen JJ, Woolley AM, et al. Thrombin generation assays are superior to traditional tests in assessing anticoagulation reversal in vitro. *Thromb Haemost* 2008; 100: 350–355.
4. Wolzt M, Weltermann A, Nieszpauro-Los M, et al. Studies on the neutralizing effects of protamine on unfractionated and low molecular weight heparin (Fragmin) at the site of activation of the coagulation system in man. *Thromb Haemost* 1995; 73: 439–443.
5. Holst J, Lindblad B, Bergqvist D, et al. Protamine neutralization of intravenous and subcutaneous low-molecular-weight heparin (tinzaparin, Logiparin). An experimental investigation in healthy volunteers. *Blood Coagul Fibrinolysis* 1994; 5: 795–803.
6. Crowther M, Lim W. Low molecular weight heparin and bleeding in patients with chronic renal failure. *Curr Opin Pulm Med* 2007; 13: 409–413.
7. Brophy DF, Martin EJ, Gehr TW, et al. Thrombin generation time is a novel parameter for monitoring enoxaparin therapy in patients with end-stage renal disease. *J Thromb Haemost* 2006; 4: 372–376.
8. Bijsterveld NR, Moons AH, Boekholdt SM, et al. Ability of recombinant factor VIIa to reverse the anticoagulant effect of the pentasaccharide fondaparinux in healthy volunteers. *Circulation* 2002; 106: 2550–2554.
9. Lisman T, Bijsterveld NR, Adelmeijer J, et al. Recombinant factor VIIa reverses the in vitro and ex vivo anticoagulant and profibrinolytic effects of fondaparinux. *J Thromb Haemost* 2003; 1: 2368–2373.
10. Young G, Yonekawa KE, Nakagawa PA, et al. Recombinant activated factor VII effectively reverses the anticoagulant effects of heparin, enoxaparin, fondaparinux, argatroban, and bivalirudin ex vivo as measured using thromboelastography. *Blood Coagul Fibrinolysis* 2007; 18: 547–553.
11. Brophy DF, Carr ME, Jr., Martin EJ, et al. The pharmacokinetics of enoxaparin do not correlate with its pharmacodynamic effect in patients receiving dialysis therapies. *J Clin Pharmacol* 2006; 46: 887–894.
12. Gerotziakas GT, Depasse F, Chakroun T, et al. Comparison of the effect of fondaparinux and enoxaparin on thrombin generation during in-vitro clotting of whole blood and platelet-rich plasma. *Blood Coagul Fibrinolysis* 2004; 15: 149–156.
13. Gerotziakas GT, Petropoulou AD, Verdy E, et al. Effect of the anti-factor Xa and anti-factor IIa activities of low-molecular-weight heparins upon the phases of thrombin generation. *J Thromb Haemost* 2007; 5: 955–962.
14. Gerotziakas GT, Elalamy I, Depasse F, et al. In vitro inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct factor Xa inhibitor rivaroxaban. *J Thromb Haemost* 2007; 5: 886–888.