Fondaparinux reversal with activated prothrombin complex concentrate in anesthetised bleeding rats

Gilles Corbonnois1,2*; Michèle Martin2,3*; Marie Hacquard3; Bruno Levy2,4; Paul Michel Mertes1,2; Thomas Lecompte2,3,5**; Gérard Audibert1,2**

1University Hospital of Nancy, Department of Anaesthesia and Intensive Care Medicine, Nancy, France; 2INSERM 961, Contrat Avenir INSERM, Nancy, France; 3University Hospital of Nancy, Haematology Laboratory – Haemostasis, Nancy, France; 4Hospital University of Nancy, Intensive Care Medicine, Nancy, France; 5Present address: Hôpitaux Universitaires de Genève, Service d’hématologie, Département académique de Médecine Interne, Faculté de médecine, UniGe, Genève, Switzerland

Correspondence to:
Thomas Lecompte
Hôpitaux Universitaires de Genève
Service d’hématologie
Département académique de Médecine Interne
Faculté de médecine, UniGe
4 Rue Gabrielle-Perret Gentil
CH-1211 Genève 14, Switzerland
Tel.: +41 223723958, Fax: +41 223727288
E-mail: thomas.pierre.lecompte@hcuge.ch
*Equal contribution as first authors.
**Equal contribution as supervisors.

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Dear Sir,
Fondaparinux (FDX) is used for prevention and treatment of venous thrombo-embolism and anticoagulation during acute coronary syndromes (1, 2). In case of bleeding and of emergency, a prompt and efficient reversal would be useful (3). There is currently none, although an antidote was designed (4).

In humans a few studies only investigated the reversal of FDX with haemostatic agents in vitro and ex vivo (5-7). In healthy volunteers receiving FDX, recombinant factor VIIa (rFVIIa) was found to have some effect on laboratory tests, but other haemostatic agents are potentially efficacious and deserve to be studied as well (8, 9).

The best criterion of reversal is bleeding (in vivo), but in humans it is barely conceivable to induce a clinically relevant bleeding. Thus, despite their limitations, animal studies are useful. We designed a randomised controlled blinded study in rats to compare the ability of rFVIIa and activated prothrombin complex concentrate (APCC), which contains activated FVII and the clotting factors of the prothrombin complex concentrate (PCC), to reverse high dose FDX in a rat arterial bleeding model (9). We also studied tranexamic acid (TXA), an antifibrinolytic drug readily available in clinical practice.

Male Wistar rats were treated according to the ethic rules of the INSERM and the European directive no. 86/609/CEE. The laboratory accreditation number was 54-74. Rats (500 ± 50g body weight) were anesthetised with isoflurane and received intravenously a high dose (400 µg/kg) of FDX. A tail transection was performed (10). Two minutes (min) later the haemostatic drug, APCC (100 IU/kg), rFVIIa (200 µg/kg), or TXA, (100 mg/kg), or saline, was administered (10 animals per group). The primary criterion was bleeding duration...
(BD) – maximal observation: 30 min. Mean arterial pressure (MAP) variation assessed the effect of blood loss on haemodynamics. To study thrombin generation (TG), calibrated automated thrombography (CAT) was performed (11, 12). Data were collected and analysed with GraphPad Prism software® version 5.01. (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as mean ± SD or median and range as appropriate. The statistical significance of FDX versus control on BD was assessed using Mann-Whitney U-test. The differences in BD between the three haemostatic agents and the two other groups were analysed with Kruskal-Wallis test, followed by Dunn’s test. The differences between the five groups in MAP and in TG parameters (lag time and endogenous thrombin potential - LT and ETP) were analysed likewise. A p-value <0.05 was
required to reject the null hypothesis in a two-tailed test with an \( \alpha \) risk of 5%.

BD was increased by FDX from 562 seconds (s) [390–1,800] to 1,800 s [800–1,800] (p < 0.01) (Figure 1A). APCC reduced BD to 400 s (285–480) (p < 0.001), and MAP was maintained throughout the observation period (Figure 1B). Neither rFVIIa nor TXA had a statistically significant effect on these parameters, although there were fewer rats with bleeding not stopped at 1,800 s in the rFVIIa group. Regarding TG, FDX prolonged LT from 1.7 ± 0.2 to 2.3 ± 0.2 min (p < 0.01) and decreased ETP from 612 ± 115 to 230 ± 41 nM*min (p < 0.01) (Figure 2A). APCC overcorrected ETP up to 1,961 ± 910 nM*min (p < 0.001) (Figure 2B), whereas rFVIIa normalized LT without improvement of ETP (Figure 2C). Administration of TXA had no effect on TG (Figure 2D).

Rats have been widely used for the risk/benefit assessment of anticoagulants and their reversal (10, 13, 14). The rat tail transection proved to be reasonably reliable. By cutting the ten distal millimetres, we performed a transection of the mid-ventral artery (15) and explored not only primary haemostasis but also coagulation.

In clinical practice, FDX action is not monitored and does not impact routine clotting assays (16). TG has been proposed as a suitable method to study the anticoagulant effect of antithrombotic drugs (12). It is considered by some to be superior to traditional tests in assessing anticoagulation reversal (17).

A few studies suggested the ability of APCC (13, 18) to reverse FDX. We chose to study bypassing agents used in haemophilia. APCC contains activated factors in small amounts, except for FVIIa, present in larger quantities (19). FXa is protected from inhibition by antithrombin when bound to prothrombin (20) and thus is likely to be less sensitive to FDX. APCC was the only haemostatic drug we studied that fully reversed the bleeding effect of FDX.

**Figure 2: Thrombin generation (thrombograms) in plasma at three time points.** T1, T2, T3: baseline, after fondaparinux, after haemostatic drug respectively; thrombin concentration (mean ± standard deviation) every 15 s as a function of time. A) Saline; B) activated prothrombin complex concentrate; C) rFVIIa; D) tranexamic acid. Thrombogram were truncated at 30 min; the curve at T3 in panel B reached baseline at 60 min.
was likely to be due to FVIIa for the initiation of coagulation and FIX(a), FX(a) and above all prothrombin (19) for the amplification and propagation.

Thrombosis is the most serious complication of bypassing therapy. APCC and rFVIIa have a low thrombotic risk in haemophilia patients (21). The large increase in ETP with APCC may be associated with a thrombotic risk in non-haemophilia patients, and thus lower doses of APCC deserve to be studied.

rFVIIa (case reports) could be useful when facing serious haemorrhage promoted by FDX (22, 23) but not if there was an overdose (24) and rFVIIa was the most studied haemostatic agent on parameter normalisation (5, 7, 8). rFVIIa was able to normalise LT as previously reported (6-8), but we did not find a significant effect on ETP. An insufficient number of animals per group may have led to a low statistical power and thus our results do not preclude an at least partial beneficial effect of rFVIIa.

Administration of TXA had effect neither on bleeding nor (as expected) on TG. The rationale of TXA use was to limit the increased sensitivity to fibrinolysis of clots formed in the presence of an anticoagulant (23).

In conclusion we found a striking association between APCC effects on bleeding and thrombin formed \textit{ex vivo}. It seems that a procoagulant agent overcomes the haemorrhagic effect of an anticoagulant such as FDX if a certain amount of thrombin activity is generated in a reasonably short time frame. PCC (unactivated clotting factors) was shown to reduce hepatosplenic bleeding in FDX anticoagulated rabbits (25). A direct comparison between PCC and APCC is thus now warranted.

Conflicts of interest
None declared.

References