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# A Novel Blood-Sparing Agent in Cardiac Surgery? First In-Patient Experience with the Synthetic Serine Protease Inhibitor MDCO-2010: A Phase II, Randomized, Double-Blind, Placebo-Controlled Study in Patients Undergoing Coronary Artery Bypass Grafting with Cardiopulmonary Bypass

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**BACKGROUND:** Antifibrinolytics have been used for 2 decades to reduce bleeding in cardiac surgery. MDCO-2010 is a novel, synthetic, serine protease inhibitor. We describe the first experience with this drug in patients.

**METHODS:** In this phase II, double-blind, placebo-controlled study, 32 patients undergoing isolated primary coronary artery bypass grafting with cardiopulmonary bypass were randomly assigned to 1 of 5 increasing dosage groups of MDCO-2010. The primary aim was to evaluate pharmacokinetics (PK) with assessment of plasmatic concentrations of the drug, short-term safety, and tolerance of MDCO-2010. Secondary end points were influence on coagulation, chest tube drainage, and transfusion requirements.

**RESULTS:** PK analysis showed linear dosage-proportional correlation between MDCO-2010 infusion rate and PK parameters. Blood loss was significantly reduced in the 3 highest dosage groups compared with control ( $P = 0.002, 0.004$  and  $0.011$ , respectively). The incidence of allogeneic blood product transfusions was lower with MDCO-2010 4/24 (17%) vs 4/8 (50%) in the control group. MDCO-2010 exhibited dosage-dependent antifibrinolytic effects through suppression of D-dimer generation and inhibition of tissue plasminogen activator-induced lysis in ROTEM analysis as well as anticoagulant effects demonstrated by prolongation of activated clotting time and activated partial thromboplastin time. No systematic differences in markers of end organ function were observed among treatment groups. Three patients in the MDCO-2010 groups experienced serious adverse events. One patient experienced intraoperative thrombosis of venous grafts considered possibly related to the study drug. No reexploration for mediastinal bleeding was required, and there were no deaths.

**CONCLUSIONS:** This first-in-patient study demonstrated dosage-proportional PK for MDCO-2010 and reduction of chest tube drainage and transfusions in patients undergoing primary coronary artery bypass grafting. Antifibrinolytic and anticoagulant effects were demonstrated using various markers of coagulation. MDCO-2010 was well tolerated and showed an acceptable initial safety profile. Larger multi-institutional studies are warranted to further investigate the safety and efficacy of this compound. (Anesth Analg 2014;119:16–25)

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**B**leeding complications and subsequent transfusion of allogeneic blood products constitute important clinical problems in cardiac surgery associated with increased morbidity and mortality.<sup>1–3</sup> The etiology of bleeding after cardiac surgery performed with cardiopulmonary bypass (CPB) is complex and involves activation of coagulation and fibrinolysis, generation of nonhemostatic thrombin, and initiation of a systemic inflammatory response.<sup>4</sup> In addition, procedural factors such as hemodilution and hypothermia as well as perioperative use of platelet inhibitors cause imbalance to the hemostatic equilibrium. Clinical studies have shown that thrombin generation during cardiac surgery, especially at the initiation of CPB and during myocardial reperfusion, may contribute to myocardial

damage and impaired hemostasis.<sup>5</sup> Therefore, inhibition of thrombin generation might provide additional benefits besides reducing transfusion requirements.

Effective and safe pharmacological interventions to reduce bleeding complications are limited. Aprotinin, a nonspecific serine protease inhibitor, was widely used for 2 decades to reduce bleeding and allogeneic transfusions in cardiac surgery but was withdrawn from the market in 2007 because of suspected increased mortality in 1 particular study.<sup>6</sup> The only currently available antifibrinolytic drugs are the lysine analogues (epsilon-aminocaproic acid and tranexamic acid), which have also been questioned with respect to their safety profile.<sup>7-9</sup> The nonspecific protease inhibitor aprotinin inhibits all serine proteases dosage-dependently; in contrast, the lysine analogues are specific inhibitors of plasminogen/plasmin without any other direct inhibitory properties. Recently, efforts have been made to develop new compounds with strong antifibrinolytic efficacy, as well as additional favorable properties with respect to coagulation and inflammation, that may not compromise patient safety.

MDCO-2010 is a synthetic small molecule, acting as an active site inhibitor of plasmin and plasma kallikrein, both of which are considered to be pivotal in mediating impaired hemostasis during and after CPB.<sup>10</sup> In addition, MDCO-2010 is an inhibitor of coagulation factors Xa, Xia, and activated Protein C.<sup>11,12</sup> It has the potential to mitigate both excessive fibrinolysis and thrombin generation during cardiac surgery. MDCO-2010 competitively inhibits the amidolytic activity of human serine proteases with the strongest affinity for plasma kallikrein and plasmin. Compared with aprotinin, the compound is substantially more potent against plasma kallikrein and factors Xa and XIa, but both inhibitors are much less effective against thrombin.<sup>12</sup> Although aprotinin has demonstrated superior efficacy in comparison to lysine analogues,<sup>13</sup> it is of biologic origin and has antigenic properties.<sup>14</sup> MDCO-2010 is a serine protease inhibitor with comparable mode of action as aprotinin, but as a synthetic small molecule, it is unlikely to result in allergic reactions.

The aim of this phase II, double-blind, placebo-controlled study was to evaluate pharmacokinetics (PK) with assessment of plasmatic concentrations, initial safety, and tolerance of increasing dosages of MDCO-2010 in cardiac surgical patients. Secondary end points included influence of MDCO-2010 on coagulation, chest tube drainage, and transfusion requirements.

## METHODS

After receiving approval from the Swiss regulatory authority (SwissMedic, No. 2010DR2212) and the regional ethics committee (Kantonale Ethikkommission Bern, No. 111/10), written informed consent was obtained from all participating patients before study enrollment. The trial was registered at ClinicalTrials.gov NCT01535222. Between November 2010 and May 2011, 34 patients scheduled for isolated primary coronary artery bypass grafting (CABG) surgery at a single center (University Hospital Berne, Switzerland) were asked to participate in the study, and all but one agreed. After induction of anesthesia, 1 patient was found to have clinically relevant ischemic mitral regurgitation requiring mitral annuloplasty.

This patient was withdrawn from the study before dosing and not included in any of the safety or efficacy analyses.

Men or postmenopausal women were enrolled if the scheduled operation was elective and performed with CPB. Exclusion criteria were previous cardiac surgery, body weight <55 kg or >110 kg, major surgical procedures within 30 days of entry, placement of a drug-eluting stent within 12 months or of a bare-metal stent within 6 weeks, left ventricular ejection fraction <35%, preoperative coagulation abnormalities (platelet count <100,000/mm<sup>3</sup> or international normalised ratio >1.5 or activated partial thromboplastin time (aPTT) >1.5 × upper limit of normal), refusal of allogeneic blood transfusion, preoperative hemoglobin <11 g/dL for men patients or <10 g/dL for women patients, administration of thienopyridines within 5 days of surgery, creatinine clearance <60 mL/min, history of stroke or transient ischemic attack within 3 months of surgery, known heparin-induced thrombocytopenia, known history of thrombophilia (e.g., deep vein thrombosis or pulmonary embolism), allergic condition, active liver disease, and any condition requiring chronic immunosuppressive medication.

## Patient Management

Patients were treated in 5 sequential cohorts randomized by a computer-generated list to blocks of 4 patients for cohort 1 and 2 and 8 patients for cohorts 3 to 5, receiving either MDCO-2010 or placebo (normal saline) in a ratio of 3:1 within each cohort. The starting dosage of MDCO-2010 in this study was based on dosages considered safe in a randomized, placebo-controlled, phase I study in healthy volunteers (unpublished data), and the highest dosage was expected to result in plasma concentrations approximately 900 nmol/L.<sup>11</sup> Blinded study medication was prepared by the local pharmacy. After completion of each cohort, a Safety Surveillance Team composed of clinical experts reviewed blinded safety and PK data and determined whether it was safe to escalate to the next dosage level. The randomization algorithm resulted in 6 treatment groups: 5 groups with escalating dosages of MDCO-2010 (MDCO-2010 group 1–5) and 1 control group (saline placebo). A doubling of dosages was planned per each new escalating dosage cohort; however, dosage adjustment was permitted as part of the protocol. The Safety Surveillance Team decided to reduce the dosage escalation to cohort 4 and 5, respectively, based on higher than expected plasma concentrations of MDCO-2010 in the first 3 study groups.

Study drug was administered via a central venous catheter with an initial loading dosage followed by a continuous infusion until sternal closure. As a measure of safety, drug infusion was started only, but immediately, after heparinization; since in this first-in-patient study, a possible procoagulant effect could not be excluded. Patients randomized to MDCO-2010 received initial loading dosages of 0.005, 0.011, 0.027, 0.047, or 0.094 mg/kg, followed by continuous infusion of 0.0125, 0.025, 0.0625, 0.109, or 0.219 mg/kg/h, respectively. In addition, 0.02, 0.04, 0.09, 0.15, and 0.31 mg MDCO-2010, respectively, were added to the pump prime fluid. Patients in the control group received equal volumes of saline in a blinded fashion.

All procedures were performed according to a standardised surgical protocol using a minimised extracorporeal circulation system (MECC, Jostra AG, Hirrlingen, Germany) with a priming volume of 600 mL (Ringer's lactate solution, Sintetica-Bioren SA, Couvet, Switzerland) in which 1 g calcium was added (Calcium-Sandoz, Sandoz, Cham, Switzerland). Surgery was performed under mild hypothermia of 32°C to 34°C, via midline sternotomy, and the left internal mammary artery was used as bypass conduit in all study patients. Antegrade cardioplegia was given via the aortic root (100 mL Cardioplexol®, Bichsel Laboratory, Interlaken, Switzerland). Cardiomyotomy suction (SmartSuction, Cardiosmart AG, Muri, Switzerland) was applied during CPB. Remaining blood from the operating field and the extracorporeal circuit was washed using a cell saver device (Autolog, Medtronic, Minneapolis, MN) and retransfused to the patient throughout the duration of the operation. All patients received a standardised anesthesia regimen using sufentanil, midazolam, and isoflurane.

For CPB, unfractionated porcine mucosa heparin (Liquemin®; Roche, Basel, Switzerland) was administered IV at a dosage of 400 units/kg, with 10,000 units added to the priming of the CPB circuit to achieve a target activated clotting time (ACT) of >480 seconds. Heparinization was controlled by the kaolin ACT every 20 minutes with the Medtronic ACT Plus® System (Medtronic, Minneapolis, MN). An additional bolus of 125 U/kg of heparin was applied if the kaolin ACT was <400 seconds during CPB. At the end of CPB, heparin reversal was accomplished with protamine chloride (1:1 ratio of the total heparin dosage). Sufficient reversal of heparin by protamine was controlled using the Hepcon® HMS Plus (Medtronic, Minneapolis, MN).

Intraoperative administration of tranexamic acid or  $\epsilon$ -aminocaproic acid was prohibited by the protocol, but it was not excluded during the postoperative period. Colloids and crystalloids were administered at the discretion of the attending anesthesiologist. Packed red blood cells were transfused if the hemoglobin was <7 g/dL during CPB. If after CPB hemoglobin concentration was between 7 and 10 g/dL, the decision to transfuse allogenic red blood cells was based on clinical signs of anemia. Fresh frozen plasma was given if the prothrombin time was >1.5  $\times$  upper limit of normal, and signs of increased bleeding were evident; the criteria for transfusion of platelets was a platelet count <100,000 mm<sup>3</sup> and suspected loss of platelet function. Both fresh frozen plasma and platelets were only administered if excessive chest tube drainage (>200 mL/h) was recorded.

Postoperatively, a standardised treatment protocol was applied. The patients' lungs were ventilated in the intensive unit until the patients were normothermic and hemodynamically stable. Blood loss via chest tubes was determined at 6, 12, and 24 hours postoperatively. Indication for surgical reexploration was driven by clinical judgment and blood loss >200 mL/h for 2 consecutive hours. Massive transfusion was defined as transfusion of  $\geq 10$  units of red blood cells within 24 hours postoperatively or the need for surgical reexploration because of hemorrhage.

## Blood Sampling

Blood samples were drawn from the arterial or central venous line at 13 time points: before heparin administration (T1), before CPB initiation (T2), 15, 30, and 60 minutes after CPB start (T3, T4, and T5), 10 minutes after protamine administration (T6), at sternal closure (T7), 15 and 30 minutes, and 4 hours after sternal closure (T8, T9, and T10), and on days 1, 2, and 4 after surgery.

Citrated blood was drawn and directly centrifuged (3000g, 10 minutes). The plasma was frozen immediately in multiple aliquots and stored at -80°C until assayed. D-Dimer levels were assessed using the D-Dimer PLUS immunoturbidometric assay (Dade Behring, Marburg, Germany; reference range: 0.06–0.25 mg/L). Thrombin activation, determined by the formation of prothrombin fragment 1+2 (F1+2), was measured using a microtiter plate-based sandwich immunoassay (Enzygnost F1+2, monoclonal, Dade Behring, Germany; reference range 69–229 pmol/L). The aPTT was determined with a Pathromtin SL reagent (Dade Behring, Marburg, Germany; reference range: 25–37 seconds). Cardiac troponin T was measured on Elecsys 2010 analyzer with an enzyme immunoassay based on electrochemiluminescence (Roche Diagnostics, Rotkreuz, Switzerland; lower detection limit 0.01 ng/mL). MDCO-2010 plasma concentrations were determined using a validated method with liquid chromatography-tandem mass spectrometry detection.<sup>12</sup> All measurements were performed immediately after thawing the plasma.

Influence on whole blood clotting was assayed with a multichannel ROTEM® analyzer (Tem Innovations GmbH, Munich, Germany) using EXTEM and INTEM reagent to activate coagulation. Fibrinolysis in whole blood was studied using a modified ROTEM analysis using EXTEM reagent with added recombinant tissue plasminogen activator (Actilyse®, Boehringer Ingelheim, Germany; final concentration of 100 U/mL in the test tube), allowing measurement of lysis onset time, defined as the time needed for clot firmness to decrease by 15% of maximum clot firmness.<sup>12</sup> A total of 140 mL blood was drawn for study purposes.

## Safety Evaluation

Patients were carefully monitored for adverse events (AE) and serious adverse events (SAE). The study included evaluations of safety, thrombotic, PK, and pharmacodynamic end points. Safety end points included a summary of all AEs and SAEs, as well as vital signs (arterial blood pressure, heart rate, and body temperature), and clinically significant changes in laboratory markers of hematology, chemistry, and coagulation. In addition to routine variables, clinical laboratory assessment included markers of histamine release and complement activation, as well as interleukin (IL)-6 and IL-10. For all AEs and SAEs, the causal relationship to study medication was assessed by the investigators. Thrombotic end points were defined as thrombotic or embolic events or laboratory signs of hypercoagulability. Early graft patency was examined intraoperatively before pericardial closure using transit time flow measurement (CardioMed, Medi.Stim, Norway). Periprocedural myocardial infarction (MI) was defined as creatine kinase MB (CK-MB)  $\geq 5$  times local laboratory upper limit of normal

and new pathological Q waves or new left bundle branch block, or imaging evidence of new loss of viable myocardium.<sup>15</sup> Acute kidney injury was defined according to the RIFLE criteria.<sup>16</sup>

### Statistical Analysis

Statistical analysis was performed using the SAS/STAT® software (SAS Institute Inc., Cary, NC). If not otherwise stated, distributions of quantitative data were described by reporting the median with 25th to 75th percentiles. Categorical variables were reported as absolute numbers and percentages. The Fisher exact test was used to investigate differences in the frequency distribution of critical events among the dosing groups. To assess intergroup differences at single predetermined time points, the Wilcoxon rank-sum test was used. PK analysis of plasma concentration versus time and the relationship between maximum plasma concentration and area under plasma concentration-time curve (AUC) as well as pharmacodynamic markers were evaluated using Phoenix WinNonlin Version 6.2 (Pharsight, Mountain View, CA). Linear (constant or linear)  $I_{max}$ , inhibitory  $I_{max}$ , and sigmoid inhibitory  $I_{max}$  models were applied to the data. All statistical tests were performed 2-sided, and the level of statistical significance was set to  $P < 0.05$ . Because this was a phase 2 study with the primary aim of evaluating PK, short-term safety, and tolerance of MDCO-2010, no  $P$  value adjustment was applied for multiple comparisons.

### RESULTS

Twenty-four patients received MDCO-2010, and 8 patients received placebo. Demographics and procedural variables are summarised in Table 1. No clinically significant differences among the 6 study groups were observed with respect to patient demographics, clinical characteristics at baseline, and operative variables. The total heparin dosage administered during surgery did not differ significantly among the study groups (Table 1).

### Pharmacokinetic Results

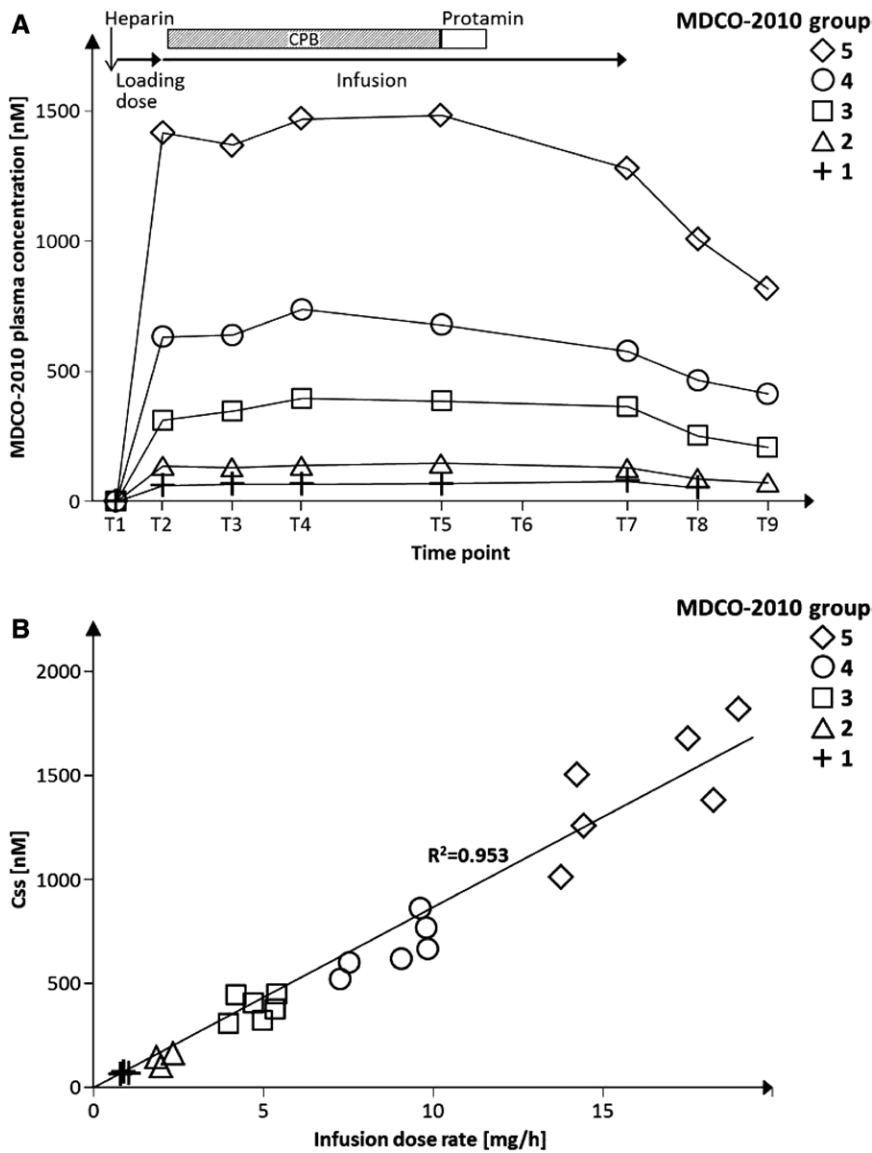
All 24 subjects in the MDCO-2010 treatment groups were included in the PK analysis. MDCO-2010 plasma levels increased rapidly with the start of bolus infusion and remained stable during the infusion (Fig. 1A). Mean ( $\pm$  standard derivation [SD]) steady-state plasma concentrations ( $C_{ss}$ ) in the escalating dosing groups were  $50 \pm 4$ ,  $96 \pm 20$ ,  $268 \pm 40$ ,  $479 \pm 102$ , and  $1009 \pm 204$  ng/mL, respectively. After the end of the infusion, MDCO-2010 plasma levels decreased approximately 40% in the first 30 minutes and had a mean ( $\pm$  standard error [SE]) terminal half-life of 81 ( $\pm 7$ ) minutes. Total body clearance decreased across the 5 dosage groups from  $220 \pm 78$  to  $153 \pm 19$  mL/min. The steady-state volume of distribution was larger than central blood volume. Systemic exposure parameters  $C_{ss}$  and  $AUC_{inf}$  increased dosage proportionally. There was a significant linear correlation between  $C_{ss}$  and infusion rate of MDCO-2010 with a Pearson  $r$  of 0.98 [95% CI, 0.95–0.99] (Fig. 1B).  $AUC_{inf}$  values were

**Table 1. Patient Characteristics and Operative Variables**

	Control group	MDCO-2010 groups					All patients
	(n = 8)	1 (n = 3)	2 (n = 3)	3 (n = 6)	4 (n = 6)	5 (n = 6)	(n = 32)
Age, y	68.5 (60; 70)	62.0 (52; 76)	65.0 (50;71)	62.0 (55; 65)	69.0 (63; 77)	65.0 (52; 73)	64.5 (59; 71)
Female sex, n (%)	0	0	0	0	2 (33)	3 (50)	5 (15.6)
Body mass index, kg/m <sup>2</sup>	23.7 (22;27)	26.8 (21; 27)	27.9 (25; 29)	26.7 (25; 28)	27.2 (26; 29)	26.7 (25; 29)	26.6 (24; 28)
Diabetes, n (%)	0	0	0	1(17)	2 (33)	2 (33)	5 (16)
Hypertension, n (%)	6 (75)	3 (100)	2 (67)	5 (83)	5 (83)	6 (100)	27 (84.4)
Prior MI, n (%)	2 (25)	0	2 (67)	3 (50)	3 (50)	0	10 (31)
LVEF (%)	60 (60; 65)	60 (55; 74)	63 (60; 64)	64 (60; 72)	65 (60; 70)	65 (60; 65)	64 (60; 65)
CPB duration, min	67 (59; 77)	45 (44; 68)	77 (38; 79)	79 (71; 82)	56 (45; 81)	57 (38; 63)	65 (50; 79)
Cross-clamp time, min	43 (37; 56)	30 (28; 39)	53 (30; 54)	47 (43; 60)	34 (31; 43)	34 (26; 38)	39 (31; 47)
No. grafts, n	3.5 (3; 4)	3 (2; 4)	4 (2; 5)	3 (3; 4)	3 (3; 4)	3 (2; 4)	3 (3; 4)
Total heparin dosage during surgery, units	38,250 (35,750; 39,625)	35,000 (32,500; 42500)	40,000 (37,500; 47,500)	36000 (33250; 38000)	40,000 (37,500; 41,750)	32,900 (31,250; 34,700)	36,000 (32,875; 40,000)
ICU stay, h	20 (20; 22)	20 (17; 20)	21 (18; 21)	20 (18; 22)	18.5 (17; 21)	21 (20; 22)	20 (18; 21)
Hospital stay, d	7.9 (6.5; 8.0)	7.9 (5.8; 9.0)	6.1 (5.0; 6.8)	6.9 (6.0; 7.8)	7.5 (7.0; 10.9)	7.9 (6.5; 8.0)	7.4 (6.2; 8.0)
30-d mortality, n	0	0	0	0	0	0	0

Data are median (25th; 75th percentiles) unless otherwise specified.

LVEF = left ventricular ejection fraction; CPB = cardiopulmonary bypass; ICU = intensive care unit.



**Figure 1.** Pharmacokinetic data in MDCO-2010 treatment groups. A, Mean plasma concentrations. B, Steady-state concentrations ( $C_{ss}$ ) defined as mean of plasma concentrations at sampling points T4 to T7. T1 = before heparin; T2 = before cardiopulmonary bypass (CPB); T3 = 15 minutes on CPB; T4 = 30 minutes on CPB; T5 = 60 minutes on CPB; T6 = after protamine; T7 = sternal closure; T8 = 15 minutes after sterna closure; T9 = 30 minutes after sterna closure.

linearly correlated with total dosage of MDCO-2010 with a Pearson  $r$  of 0.97 [CI, 0.93–0.99].

**Coagulation and Fibrinolysis**

ACT values were prolonged in MDCO-2010 groups 3 through 5 compared with placebo (Table 2). Significantly prolonged ACT values were evident in groups 4 and 5 after full reversal of heparin with protamine (124 [113; 142] in control, 166 [149; 171] in group 4, and 181 [170; 187] seconds in group 5, respectively; both  $P < 0.05$ ), and up to 30 minutes after sternal closure (Table 2). Dosage-related prolongation of aPTT was present at sternal closure in groups 2 through 5 (control 43 [39; 47] vs 97 [94; 109] seconds in group 5;  $P < 0.05$ , Table 2), but no positive association was found between prolonged aPTT and blood loss. The aPTT values returned to normal 4 hours after sternal closure. A dosage-dependent increase of EXTEM and INTEM clotting times was found at sternal closure and 15 minutes thereafter (Table 2). Clot firmness in ROTEM analyses at 10 and

30 minutes were not relevantly influenced by MDCO-2010 (data not shown). Significant suppression of D-dimer generation was observed at sternal closure in dosage groups 4 and 5. In addition, significant dosage-dependent lysis onset time prolongation was observed in dosage groups 3 through 5 (Table 2). The course of F1,2 did not demonstrate any significant difference over time among the groups (data not shown). Anticoagulant effects of MDCO-2010 on ACT, aPTT, and EXTEM clotting times demonstrated as absolute changes between baseline measurements and corresponding values at sternal closure are summarised in Figure 2.

**Blood Loss and Transfusions**

Twelve-hour postoperative chest tube drainage was significantly reduced at higher MDCO-2010 dosages: 900 (815; 950) in the control group vs 350 (300; 370) in group 3, 350 (300; 450) in group 4, and 360 (350; 400) mL in group 5;  $P = 0.002$ , 0.004, and 0.011, respectively (Table 3). In the control group 4/8 (50%), patients received transfusion of allogeneic blood

**Table 2. Pharmacodynamic Effects on Markers of Coagulation and Fibrinolysis**

Variable	Control group (n = 8)	MDCO-2010 groups				
		1 (n = 3)	2 (n = 3)	3 (n = 6)	4 (n = 6)	5 (n = 6)
<b>ACT, s</b>						
Baseline	127 (112; 142)	111 (110; 131)	128 (109; 129)	117 (110; 123)	124 (116; 145)	108 (106; 120)
Pre-CPB	638 (570; 779)	907 (593; 999)	604 (570; 734)	760 (599; 809)	767 (719; 841)	879 (742; 999)
15 min on CPB	618 (536; 849)	745 (688; 801)	628 (570; 778)	750 (653; 999)	789 (734; 916)	958 (832; 999)
30 min on CPB	693 (566; 949)	745 (719; 771)	733 (593; 999)	999 (699; 999)	940 (665; 999)	999 (848; 999)
After protamine	124 (113; 142)	NR	118 (112; 123)	133 (119; 137)	166 (149; 171)	181 (170; 187)
Sternal closure	151 (123; 156)	132 (128; 147)	148 (130; 307)	122 (111; 130)	147 (145; 159)	187 (178; 194)
15 min after sternal closure	148 (118; 155)	130 (118; 145)	138 (97; 141)	118 (113; 132)	169 (165; 178)	167 (165; 170)
30 min after sternal closure	133 (126; 141)	130 (124; 150)	120 (118; 168)	123 (121; 126)	156 (147; 165)	159 (149; 173)
<b>aPTT, seconds</b>						
Baseline	35 (33; 38)	31 (28; 32)	35 (29; 38)	34 (32; 34)	33 (31; 42)	31 (30; 33)
Sternal closure	43 (39; 47)	42 (41; 43)	52 (45; 250)	61 (57; 71)	82 (75; 96)	97 (94; 109)
15 min after sternal closure	43 (40; 49)	41 (39; 42)	53 (50; 55)	52 (47; 58)	73 (66; 88)	85 (85; 99)
30 min after sternal closure	43 (40; 52)	43 (42; 44)	48 (42; 56)	47 (40; 59)	68 (60; 81)	78 (76; 90)
4 h after sternal closure	38 (36; 47)	39 (39; 39)	38 (37; 40)	49 (35; 49)	44 (40; 52)	41 (39; 51)
<b>INTEM CT, s</b>						
Baseline	178 (172; 188)	177 (153; 186)	161 (159; 260)	170 (159; 173)	166 (154; 186)	173 (158; 180)
After protamine	212 (182; 230)	307 (186; 402)	244 (220; 569)	263 (228; 311)	302 (297; 332)	391 (360; 432)
15 min after sternal closure	200 (192; 226)	204 (161; 250)	190 (175; 330)	228 (196; 282)	266 (247; 269)	325 (263; 369)
<b>EXTEM CT, s</b>						
Baseline	57 (55; 61)	62 (62; 112)	64 (55; 74)	66 (64; 70)	58 (54; 66)	51 (48; 55)
Sternal closure	71 (68; 84)	81 (68; 119)	83 (81; 90)	94 (88; 110)	126 (97; 131)	120 (110; 147)
<b>tPA-EXTEM LOT, s</b>						
Baseline	747 (706; 945)	713 (545; 1232)	779 (747; 854)	810 (562; 895)	937 (782; 1390)	988 (879; 1125)
Sternal closure	2470 (1943; 2707)	2925 (2607; 3243)	2480 (2348; 2611)	3271 (2772; 3490)	3515 (1949; 3600)	3600 (2532; 3600)
15 min after sternal closure	2559 (1857; 2771)	2868 (1448; 3175)	2472 (835; 3324)	3202 (2945; 3441)	3298 (2005; 3600)	3466 (2292; 3600)
<b>tPA-EXTEM Lysis</b>						
Index 30 min, %						
Baseline	0.5 (0.2; 0.5)	0 (0; 42)	3 (1; 22)	3.5 (1; 11)	5 (1; 29)	2 (1; 3)
Sternal closure	97 (81; 99)	99.5 (99; 100)	100 (92; 100)	99.5 (99; 100)	100 (91; 100)	99.5 (98; 100)
15 min after sternal closure	97.5 (77; 99)	98 (36; 100)	97 (0; 98)	100 (100; 100)	99.5 (91; 100)	99.5 (96; 100)
<b>D-Dimer, mg/L</b>						
Baseline	0.3 (0.3; 0.3)	0.4 (0.2; 0.4)	0.9 (0.5; 1.2)	0.5 (0.3; 0.6)	0.5 (0.4; 0.7)	0.3 (0.3; 0.3)
Sternal closure	2.4 (1.7; 3.9)	3.4 (2.2; 3.7)	3.9 (1.4; 5.2)	2.4 (0.9; 4.0)	2.0 (1.8; 2.1)	0.9 (0.6; 1.6)

Data are median (25th; 75th percentiles).

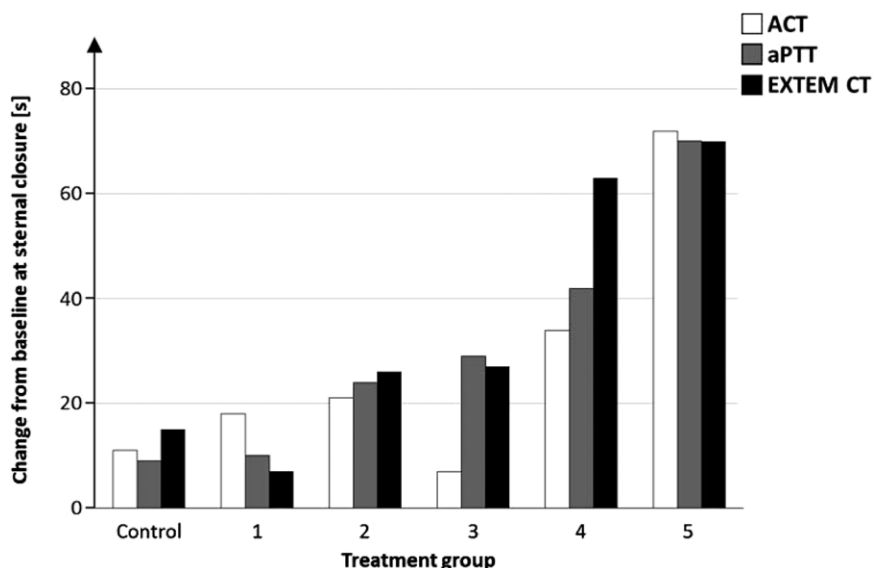
ACT = activated clotting time; aPTT = activated partial thromboplastin time, CT = clotting time; LOT = lysis onset time; NR = not recorded; CPB = cardiopulmonary bypass; tPA = tissue plasminogen activator.

products during the hospital stay compared with 4/24 (17%) in the MDCO-2010 groups. MDCO-2010 patients who received transfusions required also less volume of blood products than control patients. Hemoglobin concentrations were not different among the treatment groups during the study period. Two patients in the placebo group were treated with tranexamic acid postoperatively because of excess bleeding, but none in MDCO-2010 groups. Although still in the intensive care unit, 1 patient in the placebo group underwent local surgical revision of the saphenous vein harvest site because of diffuse bleeding. No patient in the

study underwent reoperation for any other causes. No massive transfusions occurred.

### Adverse Events and Drug Tolerance

A total of 21/24 (87.5%) patients in the MDCO-2010 groups experienced an AE, compared with 7/8 (87.5%) patients in the placebo group. In MDCO-2010 groups, 2/24 (8.3%) patients experienced an AE that was considered possibly related to study drug, compared with 2/8 (25.0%) patients in the placebo group. There were no noteworthy differences in the overall frequency of AEs among treatment groups or



**Figure 2.** Anticoagulant effects of MDCO-2010. ACT = activated clotting time, aPTT = activated thromboplastin time, and CT = EXTEM clotting time at multichannel ROTEM analyzer.

**Table 3. Blood Loss and Transfusions**

Variables	Control group (n = 8)	MDCO-2010 groups				
		1 (n = 3)	2 (n = 3)	3 (n = 6)	4 (n = 6)	5 (n = 6)
Postoperative chest tube drainage, mL						
0 to 6 h	550 (350–800)	250 (200–450)	325 (220–400)	210 (175–230)	265 (200–350)	225 (200–250)
0 to 12 h	900 (815–950)	450 (420–800)	595 (500–800)	350 (300–370) <sup>a</sup>	350 (300–450) <sup>a</sup>	360 (350–400) <sup>a</sup>
0 to 24 h	1500 (1200–1750)	885 (550–1220)	1400 (1400–1400)	630 (500–900)	1000 (800–1200)	700 (550–950)
Patients receiving transfusions, n (%)	4 (50)	0 (0)	0 (0)	1 (17)	1 (17)	2 (33)
Average no. PRBC transfusions per patient, n	2.0	0	0	0.33	0.17	0.67
Total no. blood products, n	PRBC: 16 Platelets: 5 FFP: 10	0	0	PRBC: 2	PRBC: 1	PRBC: 4

Data are median (25th–75th percentiles) unless otherwise specified.

FFP = fresh frozen plasma; PRBC = packed red blood cells.

<sup>a</sup>Post hoc analysis performed only at time point 12 hours postoperatively; *P* < 0.05 MDCO-2010 group versus control group

between the MDCO-2010 treatment groups and the placebo group (Table 4). Increases in alanine aminotransferase, bilirubin, and blood CK-MB were evenly distributed between treatment cohorts and placebo with no apparent dosage correlation associated with study drug that was consistent with other measured variables (histamine release, complement activation, IL-6, and IL-10; data not shown). None of the study patients experienced postoperative acute kidney injury.

Three patients in the MDCO-2010 treatment groups experienced 5 SAEs. One patient (MDCO-2010 dosage group 3) experienced a stroke and acute MI 7 days after the procedure and after hospital discharge. Diagnosis of heparin-induced thrombocytopenia was confirmed, and the event was classified by the investigator as unlikely related to the study drug. Another patient (MDCO-2010 dosage group 5) had an air leakage through one of the thoracic drainages that caused prolonged hospitalization. A third patient (MDCO-2010 dosage group 5) experienced vascular graft thrombosis. At the end of surgery, graft thrombosis occurred in 2 saphenous vein grafts, which showed limited flow after heparin reversal. This graft occlusion was considered possibly related to study drug. There were no deaths or withdrawals because of AEs in this study.

## DISCUSSION

Antifibrinolytic drugs have become increasingly popular in major surgery to reduce bleeding tendency. Inhibition of the fibrinolytic pathway results in decreased bleeding and consecutive need for transfusion requirements. This has been proven for both synthetic lysine analogues and aprotinin.<sup>13,17</sup> Tranexamic acid as well as epsilon-aminocaproic acid is the commonly used synthetic lysine analogues, which act by blockade of the lysine-binding sites of plasminogen, thus preventing conversion to plasmin and degradation of fibrinogen to fibrin. The nonspecific serine protease inhibitor aprotinin dosage-dependently inhibits several serine proteases and also has, thus, in addition to its antifibrinolytic activity, anticoagulant, and anti-inflammatory properties.<sup>18,19</sup> Aprotinin, widely used for >20 years in cardiac surgery, was withdrawn from the market in 2007 because of safety concerns.<sup>6</sup> However, recent revision of aprotinin's risk/benefit profile has led to its reapproval in Canada.<sup>20</sup> Because of persistent concerns about the safety of aprotinin and tranexamic acid,<sup>7,8,21</sup> it is prudent to investigate alternatives to the conventional antifibrinolytic drugs.

In this first-in-patient study, we tested the novel, synthetic, serine protease inhibitor MDCO-2010 for initial

**Table 4. Adverse Events**

System organ class	Placebo group (n = 8) n (%)	MDCO-2010 groups					All patients (N = 32) n (%)
		1 (n = 3) n (%)	2 (n = 3) n (%)	3 (n = 6) n (%)	4 (n = 6) n (%)	5 (n = 6) n (%)	
Total overall	7 (87.5)	3 (100.0)	3 (100.0)	5 (83.3)	4 (66.7)	6 (100.0)	28 (87.5)
Blood and lymphatic system disorders	0	0	0	1 (16.7)	0	0	1 (3.1)
Cardiac disorders	1 (12.5)	0	0	1 (16.7)	2 (33.3)	2 (33.3)	6 (18.8)
General disorders	4 (50.0)	2 (66.7)	2 (66.7)	3 (50.0)	1 (16.7)	2 (33.3)	14 (43.8)
Injury, poisoning and procedural complications	3 (37.5)	0	0	2 (33.3)	0	1 (16.7)	6 (18.8)
Nervous system disorders	0	0	0	1 (16.7)	0	0	1 (3.1)
Psychiatric disorders	0	0	0	0	0	1 (16.7)	1 (3.1)
Renal and urinary disorders	0	0	0	0	1 (16.7)	0	1 (3.1)
Respiratory, thoracic, and mediastinal disorders	2 (25.0)	0	1 (33.3)	1 (16.7)	0	0	4 (12.5)
Skin and subcutaneous tissue disorders	0	0	0	0	1 (16.7)	0	1 (3.1)
Surgical procedures	1 (12.5)	0	0	0	0	0	1 (3.1)
Vascular disorders	2 (25.0)	0	0	1 (16.7)	0	0	3 (9.4)

safety and tolerance. We found predictable plasmatic concentrations and an acceptable initial safety profile of the drug. Increasing dosages of MDCO-2010 resulted in higher plasma concentrations. These correlations suggest linear PK for MDCO-2010 infusion during CABG surgery in the studied range of dosing. The highest dosage group was expected to have steady-state plasma levels of 900 nmol/L, as had been demonstrated in *in vitro* experiments.<sup>12</sup> In a canine bypass model, IC<sub>50</sub> for fibrinolysis inhibition as well as reduction of bleeding tendency was determined to be 600 nmol/L.<sup>11</sup> Plasma concentrations found in the current trial were slightly higher than expected from previous *in vitro* studies.<sup>12</sup> We targeted plasma concentrations of 900 nmol/mL but reached >1000 nmol/mL in patients in the highest dosing group. Of note, this study used a small CPB circuit with a priming volume of just 600 mL. Hemodilution by higher priming volumes may result in lower plasma concentrations of MDCO-2010, if the same dosages are applied. The relatively rapid decline of plasma concentrations after the end of the infusion predicts good control but necessitates a continuous infusion of the drug.

Blood loss was significantly reduced compared with controls in groups 3 to 5, with the 3 groups showing a similar level of reduction, suggesting a plateau effect. This may indicate that the infusion of 27 µg/kg followed by 62.5 µg/kg/h combined with an additional bolus of 90 µg/kg to the priming volume (dosage group 3) is sufficient to reduce bleeding. However, the present study only included patients with a low-risk profile of bleeding complications, in whom a more pronounced reduction of bleeding with higher dosages may not have been feasible. It is not yet known whether this may also be true for patients at higher risk for bleeding complications.

ACT is widely used to monitor anticoagulation during cardiac surgery. MDCO-2010 shows dosage-dependent inhibition of kallikrein and coagulation factors XIa and Xa;<sup>12</sup> therefore, a prolongation of ACT and aPTT values after MDCO-2010 exposure was expected. Previously, comparable results have been demonstrated with the serine protease

inhibitor aprotinin, and there has been a lengthy scientific discussion whether this prolongation is a real anticoagulant effect or reflects only an artificial *in vitro* effect.<sup>19,22,23</sup> The current data demonstrate a median kaolin ACT at sternal closure of 187 (178; 194) seconds in the highest dosing group with a decline to 159 (149; 173) seconds 30 minutes thereafter. Concurrently, the aPTT values in the highest dosing group decreased from 97 (94; 109) to 78 (76; 90) seconds and were near the normal range 4 hours postoperatively: 41 (39; 51) seconds. The ROTEM results run in parallel (Table 2). All these findings demonstrate that MDCO-2010 has a moderate and real anticoagulant effect in patients. It is interesting to note that there was no association between prolonged variables of intrinsic activation of coagulation and an increased amount of postoperative bleeding. This indicates that inhibition of the intrinsic pathway did not provoke bleeding. Of note, with aprotinin, the aPTT was prolonged up to the first postoperative day compared with tranexamic acid.<sup>24</sup>

Recently, Kim et al.<sup>25</sup> investigated the *in vitro* effect of MDCO-2010 on ACT prolongation using different ACT systems and different activators: MDCO-2010 showed a concentration-dependent prolongation of ACT values regardless of the activators or the devices used for analysis. In addition, the kaolin ACT seemed to be less influenced by the drug than the celite-activated measurement. In our study, the kaolin ACT after heparin administration before CPB was 638 (570; 779) in the placebo group compared with 879 (742; 999) seconds in the highest MDCO-2010 dosing group. This 27% increase in median values corresponds to the 15% to 35% increase found in the *in vitro* study of Kim et al.<sup>25</sup> Further studies are needed to evaluate a reliable heparin management strategy and appropriate, clinically feasible monitoring when MDCO-2010 is used.

Graft patency is determined by various factors including the runoff at the distal coronary target vessel, the flow in the venous or arterial bypass graft, the quality of the surgical suture, and the balance of the coagulation/fibrinolytic state. Since the early days of antifibrinolytic therapy, there have been concerns regarding impaired graft patency.<sup>26,27</sup>



One patient in the highest dosing group experienced graft thrombosis of the venous bypass grafts after triple CABG that was classified by the investigator as possibly related to study drug. In this patient, the runoff to the right coronary artery was limited by size and quality of the target vessel, and there was no flow in the graft before sternal closure. Reevaluation of the graft revealed thrombosis; however, even after revision, there was no flow on this graft. Postoperatively, the target vessel was treated by a percutaneous intervention but without success. The patient experienced a perioperative MI. All other types of AEs were similarly distributed among the study groups.

This study has some limitations. First, because it was an initial safety and dosage-finding study to determine PK, the number of patients was too small to substantiate a significant influence on bleeding, transfusion, and AEs. However, the results demonstrated that MDCO-2010 seems to have the potential to reduce bleeding after cardiac surgery. Second, we included only low-risk patients in this study, which is likely not the population that will benefit most from intensified antifibrinolytic therapy.<sup>28</sup> Our study population was a very homogenous class of patients, in which assessment of the plasmatic concentration could be well performed although the design of the study did not allow a full PK evaluation. Third, we did not include a control group of patients treated with other antifibrinolytics (e.g., tranexamic acid); however, efficacy was not selected as the primary end point in this phase II study.

In summary, in patients undergoing CABG surgery with CPB, MDCO-2010 provided clinically significant antifibrinolytic properties combined with measurable anticoagulatory activity. Our preliminary data from this first-in-patient study of MDCO-2010 point to clinical hemostatic efficacy with an acceptable safety profile.

Subsequently to this phase II study, a larger multicenter study has already been initiated based on the results of this trial. This double-blind, randomized study aimed to compare MDCO-2010 with both placebo and tranexamic acid (ClinicalTrials.gov NCT01530399). However, this study was discontinued in response to unexpected patient safety issues encountered during the trial. The cause of the safety issues and any potential link to the study drug are still under investigation; full publication of the results is intended after final evaluation. The future of this and similar drugs is uncertain at this point. ■■

## DISCLOSURES

**Name:** Lars Englberger, MD.

**Contribution:** This author helped design and conduct the study, analyze the data, and write the manuscript.

**Attestation:** Lars Englberger has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

**Conflicts of Interest:** Lars Englberger consulted for The Medicines Company.

**Name:** Wulf Dietrich, MD, PhD.

**Contribution:** This author helped design the study, analyze the data, and write the manuscript.

**Attestation:** Wulf Dietrich has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Conflicts of Interest:** Wulf Dietrich received research funding from CSL Behring (Marburg, Germany), reported a conflict of interest with Novo Nordisk (Bagsvaerd, Denmark), consulted for Bayer (Leverkusen, Germany), received research funding from Bayer (Leverkusen, Germany), consulted for The Medicines Company (Leipzig, Germany), and received research funding from The Medicines Company (Leipzig, Germany).

**Name:** Balthasar Eberle, MD.

**Contribution:** This author helped conduct the study, analyze the data, and write the manuscript.

**Attestation:** Balthasar Eberle has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Conflicts of Interest:** The author has no conflicts of interest to declare.

**Name:** Gabor Erdoes, MD.

**Contribution:** This author helped conduct the study, analyze the data, and write the manuscript.

**Attestation:** Gabor Erdoes has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Conflicts of Interest:** The author has no conflicts of interest to declare.

**Name:** Dorothee Keller.

**Contribution:** This author helped conduct the study.

**Attestation:** Dorothee Keller has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Conflicts of Interest:** The author has no conflicts of interest to declare.

**Name:** Thierry Carrel, MD.

**Contribution:** This author helped conduct the study, analyze the data, and write the manuscript.

**Attestation:** Thierry Carrel has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Conflicts of Interest:** The author has no conflicts of interest to declare.

**This manuscript was handled by:** Jerrold H. Levy, MD, FAHA, FCCM.

## REFERENCES

- Koch CG, Li L, Duncan AI, Mihaljevic T, Cosgrove DM, Loop FD, Starr NJ, Blackstone EH. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. *Crit Care Med* 2006;34:1608–16
- Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. *Circulation* 2007;116:2544–52
- Surgenor SD, Kramer RS, Olmstead EM, Ross CS, Sellke FW, Likosky DS, Marrin CA, Helm RE Jr, Leavitt BJ, Morton JR, Charlesworth DC, Clough RA, Hernandez F, Frumiento C, Benak A, DioData C, O'Connor GT; Northern New England Cardiovascular Disease Study Group. The association of perioperative red blood cell transfusions and decreased long-term survival after cardiac surgery. *Anesth Analg* 2009;108:1741–6
- Edmunds LH Jr, Colman RW. Thrombin during cardiopulmonary bypass. *Ann Thorac Surg* 2006;82:2315–22
- Lison S, Dietrich W, Braun S, Boehm J, Schuster T, Englhard A, Perchuc A, Spannagl M, Busley R. Enhanced thrombin generation after cardiopulmonary bypass surgery. *Anesth Analg* 2011;112:37–45
- Fergusson DA, Hébert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, Teoh K, Duke PC, Arellano R, Blajchman MA, Bussières JS, Côté D, Karski J, Martineau R, Robblee JA, Rodger

- M, Wells G, Clinch J, Pretorius R; BART Investigators. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med* 2008;358:2319–31
7. Kalavrouziotis D, Voisine P, Mohammadi S, Dionne S, Dagenais F. High-dose tranexamic acid is an independent predictor of early seizure after cardiopulmonary bypass. *Ann Thorac Surg* 2012;93:148–54
  8. Sander M, Spies CD, Martiny V, Rosenthal C, Wernecke KD, von Heymann C. Mortality associated with administration of high-dose tranexamic acid and aprotinin in primary open-heart procedures: a retrospective analysis. *Crit Care* 2010;14:R148
  9. Murkin JM, Falter F, Granton J, Young B, Burt C, Chu M. High-dose tranexamic Acid is associated with nonischemic clinical seizures in cardiac surgical patients. *Anesth Analg* 2010;110:350–3
  10. Sniecinski RM, Chandler WL. Activation of the hemostatic system during cardiopulmonary bypass. *Anesth Analg* 2011;113:1319–33
  11. Szabó G, Veres G, Radovits T, Haider H, Krieger N, Bährle S, Niklisch S, Miesel-Gröschel C, van de Locht A, Karck M. The novel synthetic serine protease inhibitor CU-2010 dose-dependently reduces postoperative blood loss and improves postischemic recovery after cardiac surgery in a canine model. *J Thorac Cardiovasc Surg* 2010;139:732–40
  12. Dietrich W, Nicklisch S, Koster A, Spannagl M, Giersiefen H, van de Locht A. CU-2010—a novel small molecule protease inhibitor with antifibrinolytic and anticoagulant properties. *Anesthesiology* 2009;110:123–30
  13. Henry DA, Carless PA, Moxey AJ, O’Connell D, Stokes BJ, Fergusson DA, Ker K. Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. *Cochrane Database Syst Rev* 2011:CD001886
  14. Dietrich W, Ebell A, Busley R, Boulesteix AL. Aprotinin and anaphylaxis: analysis of 12,403 exposures to aprotinin in cardiac surgery. *Ann Thorac Surg* 2007;84:1144–50
  15. Thygesen K, Alpert JS, White HD, Jaffe AS, Apple FS, Galvani M, Katus HA, Newby LK, Ravkilde J, Chaitman B, Clemmensen PM, Dellborg M, Hod H, Porela P, Underwood R, Bax JJ, Beller GA, Bonow R, Van der Wall EE, Bassand JP, Wijns W, Ferguson TB, Steg PG, Uretsky BF, Williams DO, Armstrong PW, Antman EM, Fox KA, Hamm CW, Ohman EM, Simoons ML, Poole-Wilson PA, Gurfinkel EP, Lopez-Sendon JL, Pais P, Mendis S, Zhu JR, Wallentin LC, Fernández-Avilés F, Fox KM, Parkhomenko AN, Priori SG, Tendera M, Voipio-Pulkki LM, Vahanian A, Camm AJ, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Morais J, Brener S, Harrington R, Morrow D, Lim M, Martinez-Rios MA, Steinhubl S, Levine GN, Gibler WB, Goff D, Tubaro M, Dudek D, Al-Attar N; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. *Circulation* 2007;116:2634–53
  16. Englberger L, Suri RM, Li Z, Casey ET, Daly RC, Dearani JA, Schaff HV. Clinical accuracy of RIFLE and Acute Kidney Injury Network (AKIN) criteria for acute kidney injury in patients undergoing cardiac surgery. *Crit Care* 2011;15:R16
  17. Ker K, Edwards P, Perel P, Shakur H, Roberts I. Effect of tranexamic acid on surgical bleeding: systematic review and cumulative meta-analysis. *BMJ* 2012;344:e3054
  18. Poston RS, White C, Gu J, Brown J, Gammie J, Pierson RN, Lee A, Connerney I, Avari T, Christenson R, Tandry U, Griffith BP. Aprotinin shows both hemostatic and antithrombotic effects during off-pump coronary artery bypass grafting. *Ann Thorac Surg* 2006;81:104–10
  19. Landis RC, Asimakopoulos G, Poullis M, Haskard DO, Taylor KM. The antithrombotic and anti-inflammatory mechanisms of action of aprotinin. *Ann Thorac Surg* 2001;72:2169–75
  20. Health\_Canada. Health Canada decision on trasylol (aprotinin). Available at: [http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/\\_2011/2011\\_2124-eng.php](http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/_2011/2011_2124-eng.php). Accessed August 22, 2012
  21. Koster A, Börgermann J, Zittermann A, Lueth JU, Gillis-Januszewski T, Schirmer U. Moderate dosage of tranexamic acid during cardiac surgery with cardiopulmonary bypass and convulsive seizures: incidence and clinical outcome. *Br J Anaesth* 2013;110:34–40
  22. de Smet AA, Joen MC, van Oeveren W, Roozendaal KJ, Harder MP, Eijssman L, Wildevuur CR. Increased anticoagulation during cardiopulmonary bypass by aprotinin. *J Thorac Cardiovasc Surg* 1990;100:520–7
  23. Hunt BJ, Segal HC, Yacoub M. Guidelines for monitoring heparin by the activated clotting time when aprotinin is used during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1992;104:211–2
  24. Dietrich W, Spannagl M, Boehm J, Hauner K, Braun S, Schuster T, Busley R. Tranexamic acid and aprotinin in primary cardiac operations: an analysis of 220 cardiac surgical patients treated with tranexamic acid or aprotinin. *Anesth Analg* 2008;107:1469–78
  25. Kim H, Szlam F, Tanaka KA, van de Locht A, Ogawa S, Levy JH. The effects of MDCO-2010, a serine protease inhibitor, on activated clotting time in blood obtained from volunteers and cardiac surgical patients. *Anesth Analg* 2012;115:244–52
  26. Cosgrove DM 3rd, Heric B, Lytle BW, Taylor PC, Novoa R, Golding LA, Stewart RW, McCarthy PM, Loop FD. Aprotinin therapy for reoperative myocardial revascularization: a placebo-controlled study. *Ann Thorac Surg* 1992;54:1031–6
  27. Alderman EL, Levy JH, Rich JB, Nili M, Vidne B, Schaff H, Uretzky G, Pettersson G, Thiis JJ, Hantler CB, Chaitman B, Nadel A. Analyses of coronary graft patency after aprotinin use: results from the International Multicenter Aprotinin Graft Patency Experience (IMAGE) trial. *J Thorac Cardiovasc Surg* 1998;116:716–30
  28. Martin J, Cheng D. Tranexamic acid for routine use in off-pump coronary artery bypass surgery: evidence base “fait accompli” or more research needed? *Anesth Analg* 2012;115:227–30