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Anticoagulation With an Inhibitor of Factors XIa and XIIa During Cardiopulmonary Bypass

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ABSTRACT

BACKGROUND Exposure of blood to polyanionic artificial surfaces, for example, during cardiopulmonary bypass (CPB), induces a highly procoagulant condition requiring strong anticoagulation. Unfractionated heparin (UFH) is currently used during CPB but can lead to serious bleeding complications or development of a hypercoagulable state culminating in life-threatening thrombosis, highlighting the need for safer antithrombotics. *Ixodes ricinus* contact phase inhibitor (Ir-CPI) is a protein expressed by *I. ricinus* ticks, which specifically inhibits both factors XIIa and XIa, 2 factors contributing to thrombotic disease while playing a limited role in hemostasis.

OBJECTIVES This study assessed the antithrombotic activity of Ir-CPI in animal contact phase-initiated thrombosis models, including CPB. The safety of Ir-CPI also was evaluated.

METHODS The authors evaluated the antithrombotic activity of Ir-CPI by using in vitro catheter-induced clotting assays and rabbit experimental models of catheter occlusion and arteriovenous shunt. During CPB with cardiac surgery in sheep, the clinical applicability of Ir-CPI was investigated and its efficacy compared to that of UFH using an uncoated system suitable for adult therapy. Taking advantage of the similar hemostatic properties of pigs and humans, the authors performed pig liver bleeding assays to evaluate the safety of Ir-CPI.

RESULTS Ir-CPI prevented clotting in catheter and arteriovenous shunt rabbit models. During CPB, Ir-CPI was as efficient as UFH in preventing clot formation within the extracorporeal circuit and maintained physiological parameters during and post-surgery. Unlike UFH, Ir-CPI did not promote bleeding.

CONCLUSIONS Preclinical animal models used in this study showed that Ir-CPI is an effective and safe antithrombotic agent that provides a clinically relevant approach to thrombosis prevention in bypass systems, including highly thrombogenic CPB. (J Am Coll Cardiol 2019;74:2178–89) © 2019 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiopulmonary bypass (CPB) is commonly required during complex cardiac surgical interventions. It is a life support procedure that exposes blood to nonphysiological shear stress, turbulences, and osmotic forces as well as interaction with polyanionic artificial surfaces, promoting undesirable contact phase coagulation. Anticoagulant drugs such as unfractionated heparin (UFH) are currently used during CPB to prevent thrombotic occlusions within the oxygenator and tubing of the extracorporeal circuit. However, UFH is frequently associated with hemorrhagic side effects because it

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targets key proteases in the coagulation cascade that initiate fibrin generation (1,2). Use of UFH can also be associated with the development of platelet-activating antibodies that induce thrombocytopenia and generate a hypercoagulable state that increases the propensity for life-threatening thrombosis (3). Furthermore, large variabilities in the response to UFH, assessed by determination of the activated clotting time (ACT), and resistance to the drug have been reported. Heparin resistance has been notably observed in patients undergoing cardiovascular operations that require CPB (4,5). Thus, the search for new anticoagulants that prevent thrombus formation without increasing hemorrhage risk is a major challenge in medicine.

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Targeting factor XII (FXII) of the contact phase or factor XI (FXI) involved in the intrinsic pathway of coagulation is an alternative to current anticoagulants that usually target zymogens or proteases belonging to the common pathway. It has been shown in animal models that FXII and FXI deficiencies/inhibition protect against thrombosis without causing spontaneous bleeding (6–11). FXII and FXI inhibitions were also reported to reduce experimental thrombus formation in primates (12–14). Moreover, there is evidence supporting a role for FXI in arterial and venous thrombosis in humans (15–17). Importantly, no bleeding tendency has been reported in patients with severe deficiencies of FXII, prekallikrein, or high-molecular-weight kininogen (18). Patients with such deficiencies do not require any form of therapy before surgery despite showing a paradoxically prolonged activated partial thromboplastin time (aPTT). In contrast, FXI-deficient patients have a relatively mild bleeding disorder. In these patients, spontaneous bleedings are rare, and bleeding typically occurs after surgery or injury and particularly affects tissues with high fibrinolytic activity (oral and nasal cavities, tonsils, genitourinary tract) (19). Therefore, these observations indicate that drugs targeting FXII or FXI would be associated with better control of hemostasis than current anticoagulants.

Ticks are well known to produce salivary substances that neutralize the hemostatic system of the host for successful blood feeding (20). Decrem et al. (21) previously characterized a serine protease inhibitor from the salivary glands of the tick *Ixodes ricinus*. This protein, called *Ixodes ricinus* contact phase inhibitor (Ir-CPI), has 1 Kunitz domain and specifically binds both factor XIIa (FXIIa) and factor XIa (FXIa) (21). In vitro, Ir-CPI was reported to prolong aPTT, showing interference with the intrinsic pathway while having no effect on prothrombin time, thrombin time, and the dilute Russell's viper venom time, 3 tests that trigger blood coagulation with activators of the extrinsic or common pathways. In reconstituted systems, Ir-CPI was shown to prevent activation of FXI and FXII. These mechanistic studies strongly suggest that Ir-CPI is a specific inhibitor of both FXIIa and FXIa. In vivo studies with rodent prothrombotic models showed that Ir-CPI inhibits the formation of both venous and arterial thrombi without disturbing the clotting balance (21).

In this study using animal models (i.e., accelerated-catheter thrombosis, arteriovenous shunt, and CPB with cardiac surgery), we assessed the clinical applicability of Ir-CPI as an antithrombotic agent when thrombosis is predominantly a contact phase-driven event. Based on these results, we deduced that Ir-CPI is safer than UFH because it can inhibit the formation of thrombi without increasing bleeding risk.

ABBREVIATIONS AND ACRONYMS

ACT = activated clotting time
aPTT = activated partial thromboplastin time
CPB = cardiopulmonary bypass
FXI = factor XI
FXIa = factor XIa
FXII = factor XII
FXIIa = factor XIIa
Ir-CPI = *Ixodes ricinus* contact phase inhibitor
SEM = standard error of the mean
IU = international unit
UFH = unfractionated heparin

METHODS

Four different animal models were used (see the Online Appendix for further details). The in vitro coronary guiding catheter-induced clotting assay and the accelerated catheter thrombosis model in rabbits were an adaptation of the method described by Yau et al. (22). The rabbit arteriovenous shunt model was adapted from Wong et al. (23).

CPB AND CARDIAC SURGERY PROCEDURE. A total of 10 anesthetized female sheep (38 to 45 kg) underwent CPB, using only uncoated material, with cardiac surgery. Cannulas inserted in the carotid artery and jugular vein were connected to a circuit that included tubing, a rotating pump, and an oxygenator with a hard-shell venous reservoir. A mechanical roller pump circulated the blood through the circuit. CPB was maintained at a full-flow systemic perfusion level. Oxygenator pressure gradients were continuously measured as rapid markers of oxygenator clotting. After pericardium and left atrium opening, a mitral chordae was replaced and a commissuroplasty of the mitral valve was performed. Venting was achieved by reducing CPB enough to fill the left atrium and ventricle. The atrium was sutured, and the CPB flow level was slowly reduced. In recovery animals, a chest tube was inserted for pleural drainage, the thorax was closed, and analgesia was provided.

For Ir-CPI-treated animals (n = 3 + 2 recovery), priming of the circuit was performed with a solution containing Ir-CPI (7.5 or 10.5 mg/l). Ir-CPI was

intravenously injected with a bolus (4.6 or 6.5 mg/kg) immediately followed by an infusion (3.2 or 4.5 mg/kg/h), just before the thoracotomy. The infusion lasted until the end of the procedure. After pericardium opening and before cannulation, comparative animals (n = 3 + 2 recovery) received an intravenous bolus of 100 IU/kg of UFH to target an ACT of 715 s (human equivalent ACT: 420 s).

For experiments that included a recovery period, animals were clinically followed-up until their sacrifice on day 7. For both nonrecovery and recovery animals, an autopsy including macroscopic and microscopic examinations was performed. For microscopic analyses, standard samples from the heart, kidney, lung, liver, and brain were submitted to histological evaluation. In addition, spleen and draining lymph nodes were analyzed in recovery animals.

LIVER BLEEDING MODEL. A sagittal subxiphoid laparotomy was performed to access the liver lobes of anesthetized pigs weighing 48 to 76 kg. Wounds of 4-mm diameter and 3-mm depth were made with biopsy punches under NaCl 0.9%, Ir-CPI, or UFH treatment. The weight of blood collected in the gauze compresses placed near the biopsies was measured. After saline treatment, animals received UFH or Ir-CPI. UFH dosage administered was 50 IU/kg or 100 IU/kg (bolus), followed 15 min later by a second half-dose bolus (50 or 25 IU/kg). Additional half-doses of UFH (50 or 25 IU/kg) were administered when required to obtain ACT of 630 or 240 s (human-equivalent ACT: 420 or 160 s, respectively). For Ir-CPI-treated animals, the investigational drug was intravenously injected with a bolus (4.6 mg/kg) followed by a continuous infusion (3.2 mg/kg/h) before the first wound was made.

STATISTICAL ANALYSIS. Normally distributed data are expressed as mean ± SEM, where n represents the number of animals (see the Online Appendix for further details).

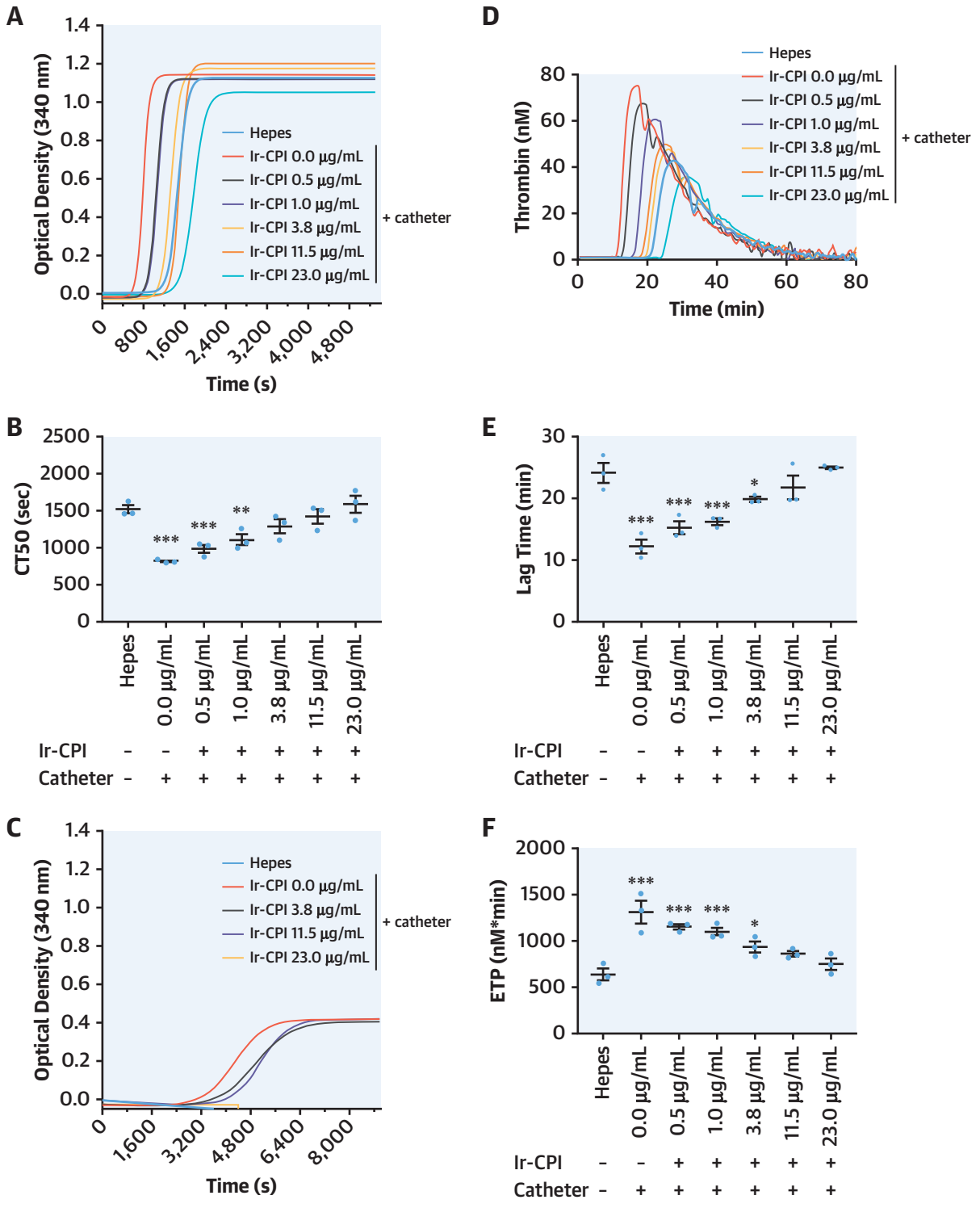
RESULTS

Ir-CPI DELAYS CATHETER-INDUCED CLOT FORMATION IN VITRO AND IN VIVO. The mean clotting time 50 (defined as the time corresponding to one-half maximal absorbance) of platelet-poor plasma was 1.9-fold shorter in the presence of catheter segments than in their absence (821 ± 12 s and 1,523 ± 55 s; n = 3; $p < 0.001$), confirming that coronary guiding catheter segments exert a prothrombotic activity (Figures 1A and 1B). Ir-CPI linearly increased the clotting time as a function of its concentration (Figure 1B). The effect of Ir-CPI on catheter-induced clotting was then explored in FXII- or FXI-deficient plasma. No catheter-induced clotting was observed in FXI-deficient plasma (data not shown). Ir-CPI decreased and even prevented (23 μg/ml) catheter-induced clotting in FXII-deficient plasma (Figure 1C). In platelet-poor plasma, thrombin generation was higher in the presence of catheter segments than in their absence (i.e., Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] condition) (Figure 1D). The presence of catheter segments shortened (2.0-fold) the time to generation of thrombin (lag time) and increased (2.1-fold) the endogenous thrombin potential (Figures 1E and 1F). Ir-CPI dose-dependently decreased the generation of thrombin and the endogenous thrombin potential, and increased the lag time in plasma incubated with catheter segments (Figures 1D to 1F).

In a rabbit model of accelerated catheter thrombosis, thrombus formation induced by repeated flushes was monitored via the increase of pressure resistance in the catheter. When compared to saline-treated animals, the time to catheter occlusion was significantly prolonged in all Ir-CPI-treated rabbits, with a minimal prolongation of 2.3-fold at 1 mg/kg (61 ± 2.5 min; n = 3; $p < 0.05$) and maximal prolongation of 4.5-fold at 18 mg/kg (117.3 ± 7.2 min; n = 3; $p < 0.05$) (Figure 2A). With doses of 1, 5, and 18 mg/kg of Ir-CPI, the plasma levels after the catheter occlusion procedure were 1.0 ± 0.3, 2.8 ± 0.6, and 9.0 ± 0.4 μg/ml (n = 3 per concentration) (Figure 2B).

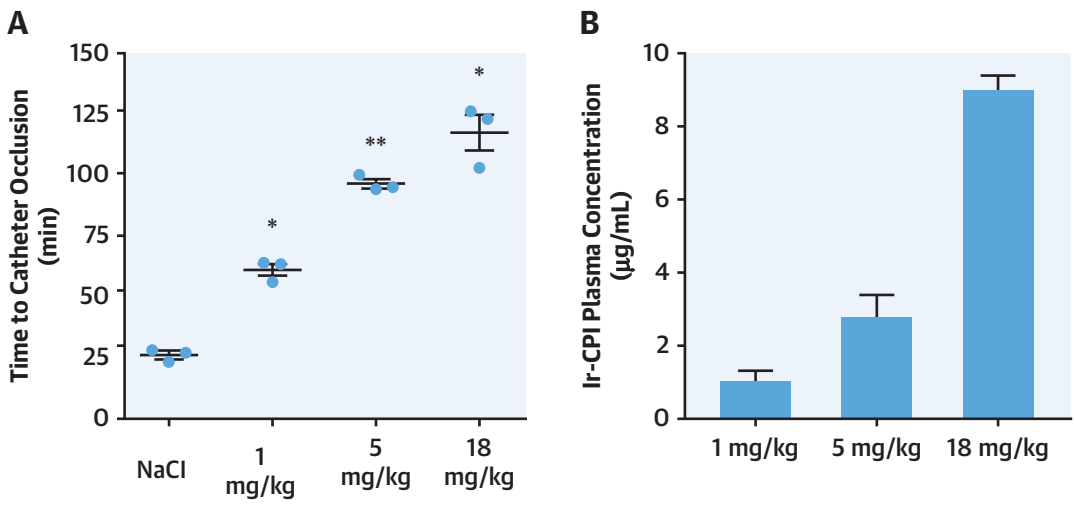
Ir-CPI INTERFERES WITH THROMBUS FORMATION IN A RABBIT ARTERIOVENOUS SHUNT MODEL. The antithrombotic action of Ir-CPI was evaluated in rabbits using an arteriovenous shunt model wherein a total inhibition of thrombus formation was obtained under UFH treatment (300 IU/kg; data not shown). In saline-treated animals (control group), the mean thrombus weight was 177.5 ± 14.1 mg (n = 6) (Figure 3A). Injection of Ir-CPI resulted in a dose-dependent and significant decrease of clot weight. In rabbits, 1, 3, and 5 mg/kg doses of Ir-CPI resulted in clot weight decreases of 37% ($p = 0.078$), 62% ($p < 0.05$), and 97% ($p < 0.001$), respectively (Figure 3A). At 5 mg/kg, 2 of 3 animals did not show any thrombus formation. At the end of the procedure (t = 40 min), the mean aPTT ratio was 0.99 ± 0.01 (n = 4) in the control group (Figure 3B). A single bolus administration of Ir-CPI increased the aPTT ratio by 25% ($p < 0.01$), 40% ($p < 0.001$), and 52% ($p < 0.001$) at 1, 3, and 5 mg/kg, respectively (Figure 3B). Injection of Ir-CPI at ≥3 mg/kg increased significantly ($p < 0.001$) the percentages of inhibition of FXII and FXI procoagulant activities

FIGURE 1 Effect of Ir-CPI on the Prothrombotic Activity of Catheters In Vitro



Catheter segments were incubated with platelet-poor plasma **(A, B)** or factor XII–deficient plasma **(C)** in wells containing increasing concentrations of Ir-CPI. Clotting was initiated by the addition of $CaCl_2$. **(B)** Time corresponding to the one-half maximal absorbance was defined as clotting time 50 (CT50). **(D)** Thrombin generation determination after incubation of catheter segments with platelet-poor plasma. **(E, F)** Lag time and endogenous thrombin potential (ETP). One-way ANOVA statistical analysis with Dunnett's multiple comparison test was used. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Hepes = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Ir-CPI = *Ixodes ricinus* contact phase inhibitor.

FIGURE 2 Effect of Ir-CPI on the Time to Catheter Occlusion in Rabbits



(A) Rabbits were given saline or Ir-CPI intravenously before the beginning of catheter flushing. Thereafter, every 5 min, blood was withdrawn into the catheter, held in a syringe, and slowly reinjected until total occlusion of the catheter. **(B)** Ir-CPI plasma concentration after catheter occlusion. Results are expressed as mean ± SEM of 3 experiments. One-way analysis of variance statistical analysis with Dunnett multiple comparison test was used. $p < 0.05$; $^{**}p < 0.01$. Ir-CPI = *Ixodes ricinus* contact phase inhibitor.

compared to the saline-treated group (Figures 3C and 3D). With an injected dose of 5 mg/kg of Ir-CPI, percentages of inhibition of FXI and FXII procoagulant activities were 44.2 ± 1.5% (n = 3) and 36.2 ± 2.1%, respectively. Mean Ir-CPI plasma levels were 0.9, 2.8, and 4.2 µg/ml at 40 min post-intravenous injection after administration of 1, 3, and 5 mg/kg of Ir-CPI, respectively (Figure 3E).

Ir-CPI CONFERS THROMBOPROTECTION DURING CPB IN CARDIAC SURGERY ON BEATING HEART. To investigate the clinical relevance of Ir-CPI in thrombosis prevention, we used CPB in cardiac surgery on beating heart. Mitral chordae replacement and commissuroplasty were performed under CPB in healthy sheep treated with Ir-CPI or UFH used as comparative drug. Time of overall procedure was between 40 to 106 min in Ir-CPI-treated sheep and 34 to 54 min in UFH-treated sheep. In untreated sheep, total obstruction of the arterial cannula at the cannulation site occurred in less than 2 min, and CPB could not be performed. In contrast, Ir-CPI and the comparative drug, UFH, prevented occlusion of the CPB system in all animals.

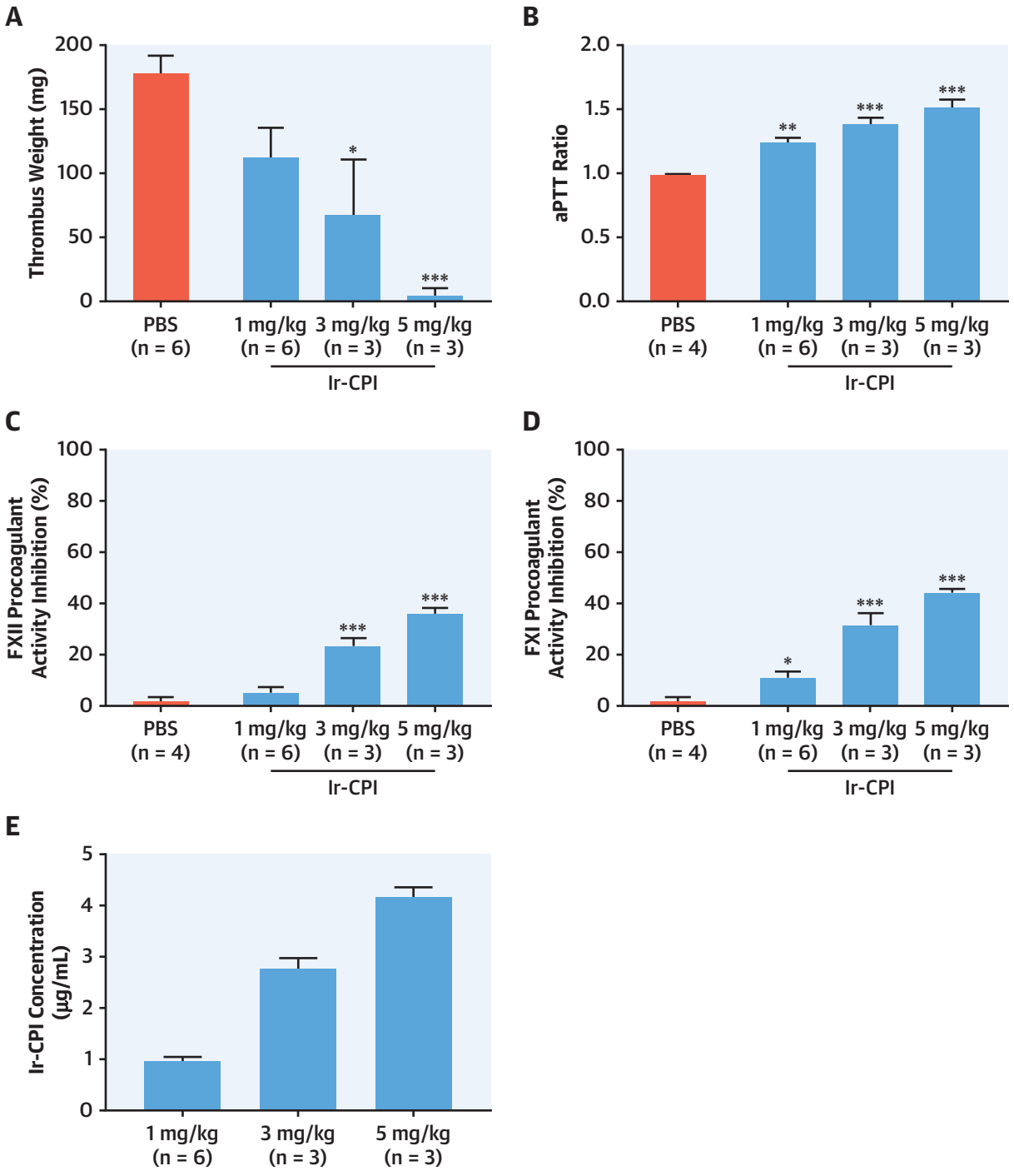
At the start of CPB, mean Ir-CPI plasma concentration was 9.4 ± 1.9 µg/ml (n = 5). Corresponding mean percentage of inhibition of FXII procoagulant activity was 83.8 ± 2.9% (n = 5). Ir-CPI plasma concentration and FXII inhibition remained stable during the overall procedures (Figures 4B and 4C).

In animals injected with Ir-CPI, pressure in the venous and arterial lines and blood pressure gradient over the oxygenator remained constant throughout the procedure, indicating no functional obstruction of the oxygenators (Figure 4B). Gas exchange parameters also remained constant during CPB. No statistical differences were observed with regard to partial arterial pressure of oxygen (Pa_{O_2}), partial arterial pressure of carbon dioxide (Pa_{CO_2}), and arterial oxygen saturation (Sa_{O_2}) values between Ir-CPI- and UFH-treated sheep during the overall procedure (Online Table 1). Sa_{O_2} reached 99% to 100% in both groups of sheep and was stable throughout the procedure, demonstrating efficient oxygenation (Online Table 1).

Standard clinical hematology and biochemistry markers showed similar modifications of values during the procedures in Ir-CPI- and UFH-treated animals. In both groups, parameters fully or partially recovered by the end of the recovery period (day 7) (Online Figures 1 to 3). Only erythrocyte and reticulocyte counts as well as hemoglobin and hematocrit concentrations took longer to recover in animals treated with Ir-CPI than in animals treated with UFH (Online Figure 4).

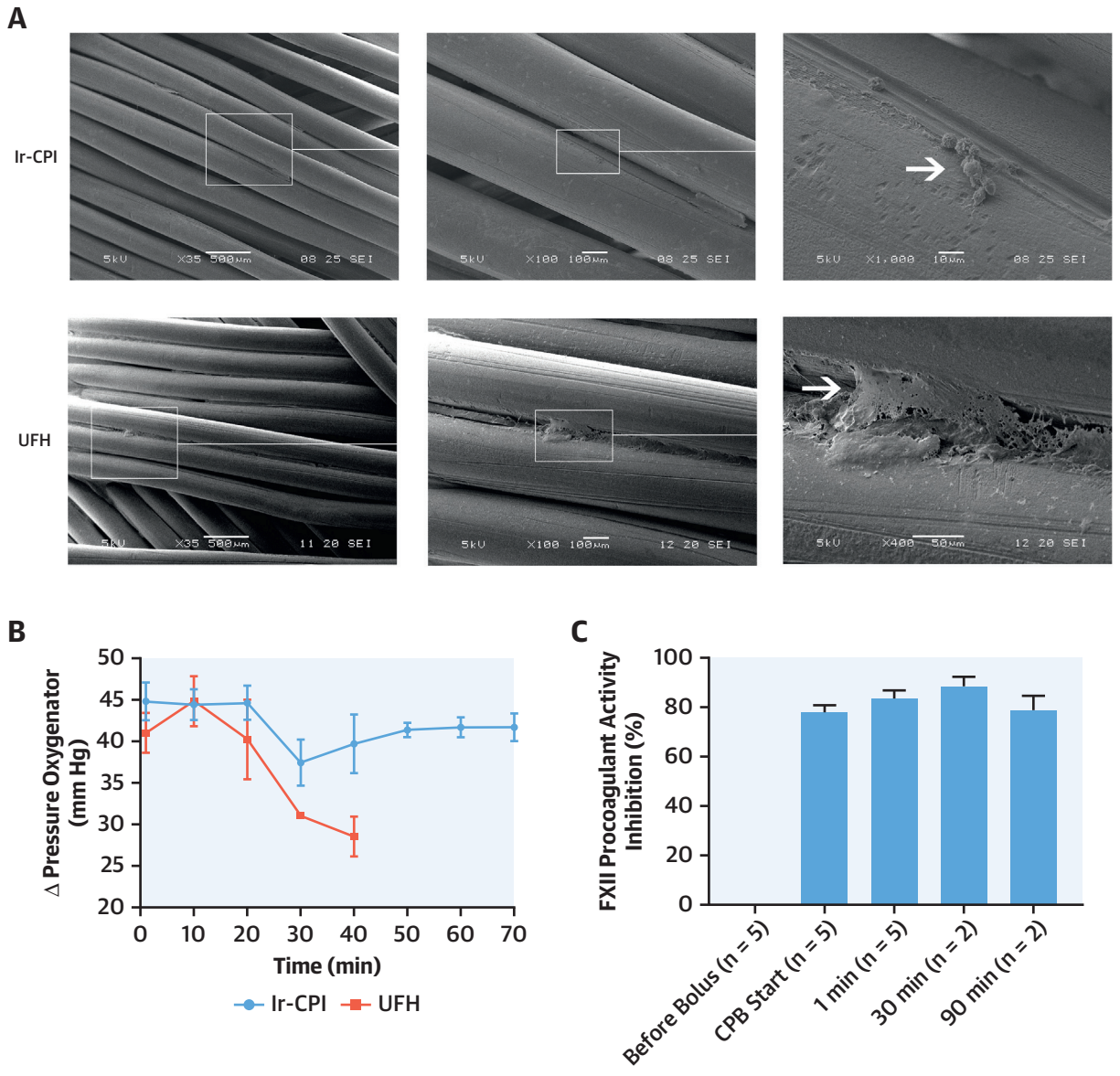
Thrombosis on oxygenator membranes was evaluated by scanning electron microscopy. Traces of fibrin were occasionally observed between oxygenator capillaries in membranes of Ir-CPI- and UFH-treated sheep. In the Ir-CPI group, rare blood cells were also observed between fibers (Figure 4A).

FIGURE 3 Effect of Ir-CPI in a Rabbit Model of Arteriovenous Shunt Thrombosis



Rabbits were intravenously treated with a bolus of saline or Ir-CPI (1, 3, or 5 mg/kg), followed by an infusion of saline 5 min before the start of shunt perfusion until the end of the procedure (t = 40 min). Thrombus weight **(A)**, aPTT ratio **(B)**, percentages of inhibition of FXII **(C)** and FXI **(D)** procoagulant activity, and Ir-CPI plasma concentration **(E)** at t = 40 min in saline- or Ir-CPI–treated animals. Data are expressed as mean ± SEM with sample size (n). One-way analysis of variance statistical analysis with Dunnett multiple comparison test was used. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. aPTT = activated partial thromboplastin time; FXI = factor XI; FXII = factor XII; Ir-CPI = *Ixodes ricinus* contact phase inhibitor; PBS = phosphate-buffered saline.

FIGURE 4 Antithrombotic Effect of Ir-CPI and UFH During CPB



Ir-CPI–treated sheep were intravenously injected with a bolus of Ir-CPI (4.6 to 6.5 mg/kg), followed by an infusion (3.2 to 4.5 mg/kg/h) 30 min before the start of CPB. Another group of animals received UFH (100 IU/kg). **(A)** Scanning electron microscopy representative images of gas-exchanging capillaries of oxygenator membranes for nonrecovery animals treated with Ir-CPI or UFH. **Arrows** point to either blood cells or fibrin deposits between fibers. Magnification ×35 to ×1,000. **(B)** Changes in blood pressure gradients over the oxygenator for animals treated with UFH or Ir-CPI. Mean ± SEM (n = 5 from 1 to 30 min and n = 2 or 3 from 40 to 70 min). **(C)** Percentage of FXII procoagulant activity inhibition (mean ± SEM). CPB = cardiopulmonary bypass; IU = international unit; UFH = unfractionated heparin; other abbreviations as in Figure 3.

Following CPB and cardiac surgery procedures, unsacrificed animals from the Ir-CPI and UFH groups had a normal recovery, and no specific clinical observations were made until their sacrifice on day 7. After macroscopic and microscopic analyses in organs of nonrecovery and recovery animals sacrificed at day 0 or day 7, respectively, neither Ir-CPI- nor UFH-related findings, including relevant inflammatory changes, were observed.

Ir-CPI DOES NOT INDUCE BLEEDING IN A PIG MODEL. The bleeding effect of Ir-CPI and UFH, used as comparative drug, was investigated in a pig model by

performing lesions with a biopsy punch in liver lobes. At the start of lesions, mean Ir-CPI plasma concentration was 8.5 ± 1.0 μg/ml (n = 4), and mean inhibition of FXII procoagulant activity was 70.9 ± 0.8% (n = 4). Ir-CPI concentration and FXII inhibition remained stable during the overall procedures (**Figure 5A**).

Blood loss weight in saline-treated control animals was 3.4 ± 0.7 g (n = 44). No statistical difference was observed with Ir-CPI-treated animals (13.2 ± 4.1 g; n = 18), whereas blood loss weight was significantly higher ($p < 0.001$) in UFH-treated animals (34.6 ± 7.8 g; n = 31; and 57.3 ± 11.7 g; n = 20; with 50 + 25 IU/kg or 100 + 50 IU/kg). Moreover, blood loss weight was statistically higher ($p < 0.01$) in animals treated with UFH (100 + 50 IU/kg) than in those treated with Ir-CPI (**Figure 5B**). Finally, bleeding was systematically observed on previously cauterized wounds under UFH treatment. This phenomenon was not observed at cauterized wound sites in Ir-CPI–treated animals.

DISCUSSION

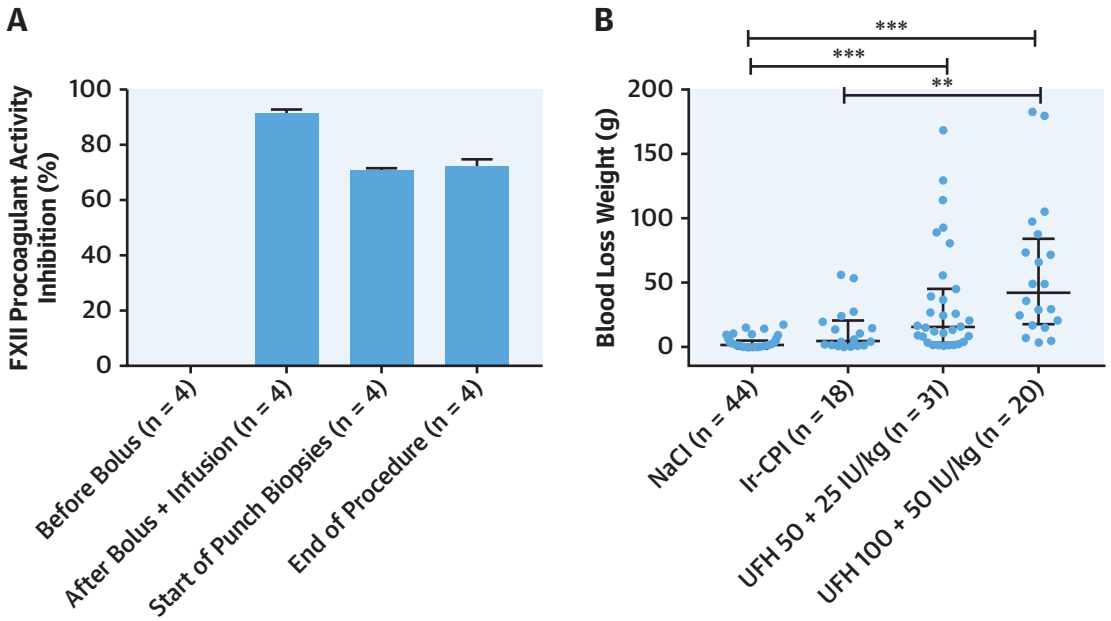
There is a medical need to find safer antithrombotic treatments in patients undergoing cardiovascular interventions. In our study, Ir-CPI was proven to be as efficient as UFH during CPB in cardiac surgery and to be a safe antithrombotic compound.

Previous studies showed that Ir-CPI specifically binds to both FXIIa and FXIa. In vitro, this molecule prolongs aPTT and inhibits thrombin generation induced by activators of the contact phase (ellagic acid and phospholipids), whereas it has no effect on prothrombin time, thrombin time, and dilute Russell's viper venom time. Ir-CPI is known to prevent activation of FXI into FXIa by FXIIa and activation of FXII into FXIIa by FXIa (21). In vivo effects of Ir-CPI were previously examined using both venous and arterial thrombosis models. Intravenous administration of Ir-CPI in rats and mice resulted in a dose-dependent reduction of arterial and venous thrombus formation (21). Moreover, treated mice were protected against collagen- and epinephrine-induced thromboembolism (21).

In this study, we evaluated the potential antithrombotic effect of Ir-CPI in preclinical contact phase-driven thrombosis models with increasing complexity. Catheters such as those used during percutaneous coronary intervention are procoagulant, and we and other investigators (22) showed that the capacity of catheter segments to promote clotting is dramatically attenuated or abrogated in both human FXI and FXII-deficient plasmas, suggesting that the prothrombotic effect of catheters depends on these key components. Using human plasma, we showed that Ir-CPI dose-dependently delays catheter-induced clot formation but also delays and reduces catheter-induced thrombin generation. Using a rabbit model of accelerated catheter thrombosis, we showed that Ir-CPI has also dose-dependent antithrombotic properties. Moreover, protection against thrombus formation by Ir-CPI was also observed when the arteriovenous shunt model was used. Decrease of thrombus weight was observed in Ir-CPI–treated animals (97% of thrombus weight reduction at 5 mg/kg). At the end of the procedure, with an injected dose of 5 mg/kg of Ir-CPI, aPTT ratio was increased by 52%, and mean percentages of inhibition of FXI and FXII procoagulant activities were 44% and 36%, respectively. Results are consistent with the antithrombotic effects documented for rHA-Infestin-4, another therapeutic protein inhibiting FXIIa, with antisense FXI or FXII oligonucleotides, and with monoclonal antibodies inhibiting either FXIIa or FXIa activity in the arteriovenous shunt model performed in various species (10,13,14,24). Nevertheless, in the latter studies, almost complete or complete abolition of thrombus formation was obtained with FXI or FXII inhibition superior to 80%. This may suggest that dual FXII and FXI inhibition offers an advantage over single clotting factor inhibition.

We further assessed the efficacy of Ir-CPI in an animal model closer to a clinical indication. Ovine and porcine models accurately simulate characteristics of human anatomy, physiology, and hemostatic properties (25). Using CPB associated with 2 cardiac procedures on beating heart, namely, mitral chordae replacement and commissuroplasty of the mitral valve performed on healthy sheep (**Central Illustration**), we showed that Ir-CPI is as efficient as UFH in preventing clot formation and maintaining physiological parameters. In this model, Ir-CPI confers its thromboprotection with a mean inhibition of FXII procoagulant activity of 84%. During the overall procedure, blood gas parameters (Pao_2, $Paco_2$, and Sao_2) remained stable, demonstrating efficient oxygenation. Pressure in the venous and arterial lines and blood pressure gradient over the oxygenator remained unchanged throughout the procedure, indicating no functional obstruction of the oxygenators. For standard hematology or biochemistry markers, modifications of values have been similarly observed in both groups, suggesting that changes were associated with the procedure (e.g., hemodilution effect) rather than the treatment. In both groups, these parameters fully or partially recovered. Nevertheless, erythrocyte and reticulocyte counts as well as hemoglobin and hematocrit concentrations took longer to recover in animals treated with Ir-CPI than

FIGURE 5 Effects of Ir-CPI and UFH on Bleeding



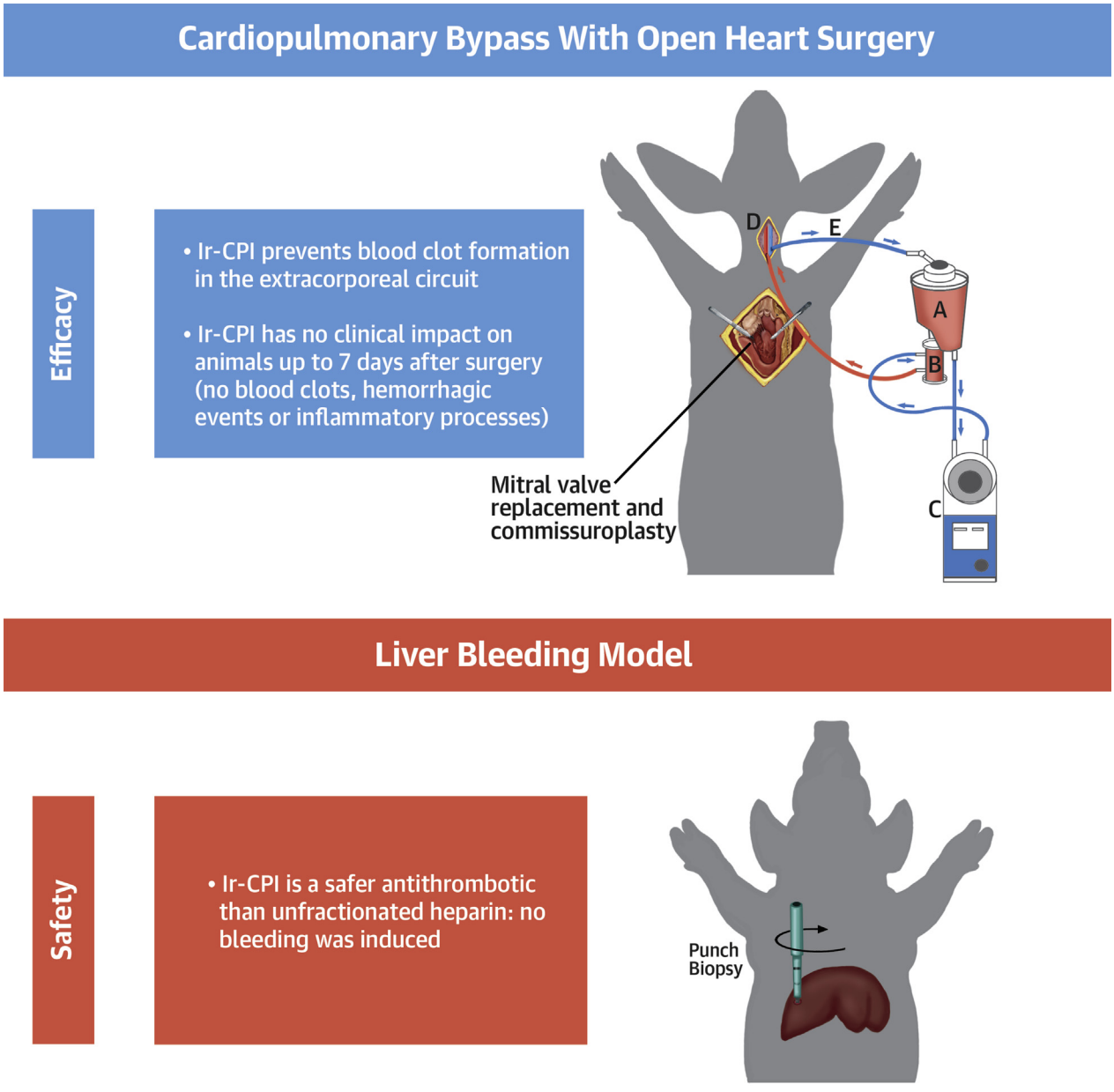
Animals were first treated with saline. Next, animals were treated with either Ir-CPI or UFH. Ir-CPI–treated pigs were intravenously injected with a bolus (4.6 mg/kg) followed by an infusion (3.2 mg/kg/h) 30 min before the first wound was made. UFH-treated pigs received either 50 IU/kg or 100 IU/kg, followed 15 min later by a second half-dose (50 or 25 IU/kg). Additional half-dose UFH administrations (50 or 25 IU/kg) were given when required to reach the target activated clotting time. **(A)** Percentage of FXII procoagulant activity inhibition. **(B)** Blood loss weight obtained until clotting of the wound or during a maximal period of 25 min in saline-, UFH- or Ir-CPI–treated animals. Data are expressed as mean ± SEM with sample size. Kruskal-Wallis statistical analysis with Dunn multiple comparison test was used. $^{**}p < 0.01$; $^{***}p < 0.001$. Abbreviations as in Figures 3 and 4.

in animals treated with UFH. This phenomenon was likely related to the high number of blood samplings performed during procedures in animals treated with Ir-CPI. Oxygenator membrane examination by scanning electron microscopy showed no differences between Ir-CPI and UFH groups in terms of protein or blood cell deposits. Full recovery was obtained after CPB and cardiac surgery with no related-Ir-CPI findings and no relevant inflammatory changes observed after organ macroscopic and microscopic analyses.

Both FXI and FXII knockdown have already been reported to be effective in preventing clotting on artificial surfaces such as catheters in rabbits (26). Furthermore, Larsson et al. (10) showed that the use of a monoclonal immunoglobulin G targeting FXIIa resulted in maintenance of physiological parameters (plasma pH, oxygen saturation) and circuit blood flow during a 6-hour ECMO-like procedure in rabbits without increasing bleeding. However, no recovery animals were included in that study, so no conclusions can be made about its efficacy and safety and thus its clinical applicability in such a procedure.

In order to simulate the worst-case scenario, the extracorporeal circuits used in our study were uncoated, thus excluding any hemocompatibility of the circuit and protection of blood activation by the material surface. Moreover, other than coagulation activation, another complication witnessed during CPB, notably with an uncoated circuit, is the inflammatory response. Indeed, exposure of a patient's blood to the nonendothelialized surface of the circuit, surgical trauma, or ischemia/reperfusion injury results in widespread activation of the innate immune system (27). Thus, in the CPB model, Ir-CPI acts in a highly inflammatory and thrombogenic environment in which the contact phase is strongly activated by exposure of blood to the surface of the extracorporeal unit, the oxygenator, pump, and connections between the animal and the extracorporeal device. Thrombin generation is also mediated by activation of the tissue factor-dependent extrinsic pathway through exposure of blood to air and tissue factor secretion in the wound. Therefore, the results indicated that Ir-CPI still is efficacious in a complex system involving both the intrinsic and extrinsic

CENTRAL ILLUSTRATION **Effect of the Contact Phase Inhibitor *Ixodes ricinus* Contact Phase Inhibitor in the Cardiopulmonary Bypass Efficacy Model, and Safety Assessment**



Pireaux, V. et al. J Am Coll Cardiol. 2019;74(17):2178–89.

A, Venous reservoir; **B**, oxygenator; **C**, heart-lung machine; **D**, arterial cannula; and **E**, venous cannula.

pathways of coagulation. This may be explained by the potential inhibitory effect of Ir-CPI on retroactivation of the contact phase by thrombin known to amplify the thrombin generation process in the presence of tissue factor (28).

Hemorrhage is the primary complication of anticoagulation therapy during CPB, even when anticoagulants are maintained within their therapeutic margin (1,2). Given that Decrem et al. (21) already showed that Ir-CPI did not induce bleeding in rodent tail transection assays, we assessed the safety of Ir-CPI in a liver bleeding assay performed in pigs (**Central Illustration**). This species is widely used for evaluation of the hemostatic performance of drugs because of

its size and similarity to humans with respect to the coagulation system (29–32). Moreover, anatomically the porcine liver is more easily accessible than the ovine liver. Using individual pigs, we showed that Ir-CPI is a safe thromboprotector with a very limited effect on bleeding, whereas UFH induced significant blood loss. Likewise, the interindividual variability in volume of blood collected was considerably higher with UFH than with Ir-CPI, as some animals presented significant bleedings whereas others had blood collections within normal limits. This observation may be of relevance to clinical findings with UFH (33).

Unlike UFH, Ir-CPI may not require neutralization when anticoagulation is no longer needed because it has a short distribution half-life (e.g., 40 min in rabbits) defining its period of activity. On the contrary, when UFH is used, administration of protamine sulfate is needed to quickly neutralize the antithrombin mediated-anticoagulant properties of UFH at the end of surgery in order to prevent any hemorrhagic events. However, clinical use of protamine is associated with safety concerns, including thrombocytopenia and potentially life-threatening anaphylactic complications with critical symptoms such as severe hypotension, cardiovascular collapse, pulmonary edema, pulmonary vasoconstriction, and pulmonary hypertension (34,35). Furthermore, prospective randomized trials have shown that protamine administration for UFH neutralization is associated with increased bleeding, particularly after cardiothoracic surgery with CPB (36,37).

STUDY LIMITATIONS. Although animal models represent the only rational approach for preclinical studies, they might not fully predict the pharmacological response to Ir-CPI exposure in humans. For example, during in vitro assays, plasma from animal species in this study presented a limited response to Ir-CPI in terms of FXII and/or FXI inhibition and/or prolongation of aPTT compared to human plasma (data not shown). Nevertheless, in vivo, Ir-CPI was efficient in inhibiting thrombus formation in all models studied.

Due to anatomic constraints, the liver bleeding assay could not be performed in the same species as that used for the efficacy model. Moreover, evaluation of the safety of molecules using documented bleeding assays does not always reflect the situation that will be encountered in humans. However, results obtained in the pig bleeding assay are consistent with data reported in the literature showing that FXII inhibition and, in some instances, FXI inhibition are not associated with a bleeding tendency (6–11). Even if tissue factor as well as FXII activation on the polyanionic surface of medical devices might trigger thrombosis during CPB, no assessment of the specific role of this factor was performed here. Although not reported to inhibit the extrinsic pathway in vitro, other experiments are required to investigate whether Ir-CPI could interfere with this pathway in vivo. Finally, as with any foreign therapeutic protein, there is a risk that Ir-CPI might be immunogenic. Nevertheless, the relevance and impact of this immunogenicity, if any, must be carefully evaluated during preclinical toxicology and safety studies.

CONCLUSIONS

To the best of our knowledge, this is the first time that an inhibitor of both FXIa and FXIIa has been shown to provide thromboprotection during CPB in cardiac surgery, to have no clinical impact on the recovery of animals, and to be safer than UFH in terms of bleeding. Such preclinical results indicate that Ir-CPI might be an important therapeutic agent by protecting patients from thrombosis in clinical situations involving activation of the contact phase but also in highly inflammatory and thrombogenic procedures involving both the intrinsic and extrinsic pathways of coagulation.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Extracorporeal circulation during CPB is associated with thrombin generation, and high-dose heparin is needed to suppress thrombus formation. In animal models of CPB and liver injury, Ir-CPI, a specific inhibitor of coagulation factors XIa and XIIa, prevents thrombosis with less hemorrhagic risk than heparin.

TRANSLATIONAL OUTLOOK: Clinical studies are needed to evaluate the safety and efficacy of Ir-CPI and other factors XI and XII inhibitors as alternatives to heparin during CPB.

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APPENDIX For an expanded Methods and Results section as well as a supplemental table and figures, please see the online version of this paper.