Ir-CPI: An Original Inhibitor of Thrombosis and Thrombosis associated with Cancer

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INTRODUCTION

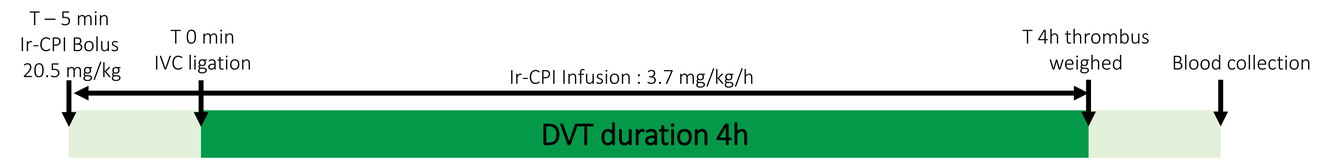
Ir-CPI, a **protein** derived from **the saliva** of **the tick** *Ixodes ricinus,* specifically binds to **FXIa** and **FXIIa**. Ir-CPI was reported to **inhibit the contact phase pathway** since it prolongs the activated Partial Thromboplastin Time (aPTT) whilst it is inactive on the prothrombin (PT) and thrombin (TT) time *in vitro*. Ir-CPI confers a dose-dependent thromboprotection in several models of venous and arterial thrombosis [1]. Thrombus formation in the mouse model of **Deep Vein Thrombosis** (DVT) associated to a partial ligature of the vena cava involves mainly the activation of FXII [2]. The mouse **arteriolar laser injury** model is strictly dependent on **Tissue Factor** (TF) and independent on FXII [3]. In mice bearing a pancreatic tumor, **a procoagulant state** is linked to the **TF** expression by **cancer cell-derived microparticles** [4].

AIM

- To evaluate the efficacy of Ir-CPI in the prevention of thrombosis dependent of the TF pathway in mouse models of thrombosis.

METHODS

- **Deep vein thrombosis model** (C57BL/6J mice): Flow restriction was induced by ligation of the inferior vena cava (IVC) below the left renal vein over a spacer (0.26 mm in diameter) that was removed after the ligation. The procedure decreases the diameter of the vascular lumen of the IVC by 90%.



- **Arteriolar laser injury model** (C57BL/6J mice): Cremaster preparation and intravital microscopy were performed as previously described [5]. Vessel wall injuries were induced with a nitrogen-dye laser. Subcutaneously injected mouse pancreatic adenocarcinoma Panc02 cells were used for thrombosis associated with cancer experiments.

RESULTS

A. Ir-CPI reduces thrombosis in a mouse model of deep vein thrombosis

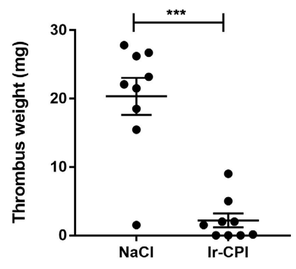


Figure 1. Effect of Ir-CPI infusion on thrombus weight in the DVT model. Weight (mg) of harvested IVC thrombi 4h after DVT induction in mouse continuously infused with saline (n=9) or continuously infused with Ir-CPI (n=9) during 4h. Dots represent individual experiments. Data represent mean ± SEM. A Mann-Whitney t test (***p<0.001) was performed. Ir-CPI plasma levels were 6.0 ± 0.7 µg/mL at the end of the experiments.

B. Ir-CPI reduces thrombus formation after laser-induced arteriole wall injury

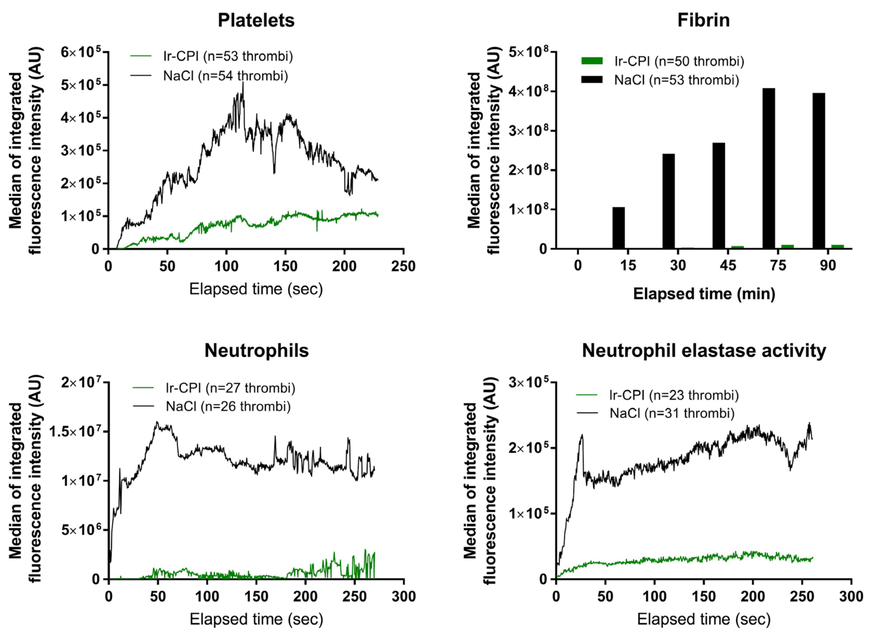


Figure 2. Effect of Ir-CPI infusion on platelet and neutrophil accumulation and fibrin generation after laser injury. Platelets, neutrophils and fibrin were respectively detected using DyLight 649-conjugated anti-GPIb Ab, PE-conjugated anti-Ly6G Ab and Alexa Fluor 488-conjugated anti-fibrin Ab. The median integrated fluorescence intensity of the initial thrombi over time is presented for platelets, neutrophils, and neutrophil elastase activity in absence or presence of Ir-CPI. For fibrin, the graph represents the median of integrated fluorescence intensity in presence or absence of Ir-CPI over time until 90 min after the laser injury. AU: arbitrary units. Ir-CPI plasma levels were 6.5 ± 1.0 µg/mL 150 min after the first laser injury (n=5).

C. Ir-CPI reduces thrombosis associated with cancer

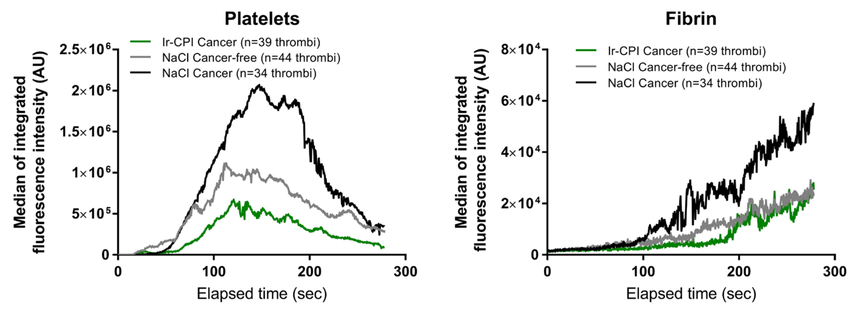


Figure 3. Effect of Ir-CPI infusion on platelet accumulation and fibrin generation after laser injury. Platelets and fibrin were respectively detected using DyLight 649-conjugated anti-GPIb Ab, Alexa Fluor 488-conjugated anti-fibrin Ab. The median of integrated fluorescence intensity over time of initial thrombi is presented. AU: arbitrary units.

D. Ir-CPI alters neutrophil activation through decreased CD11b expression *in vitro*

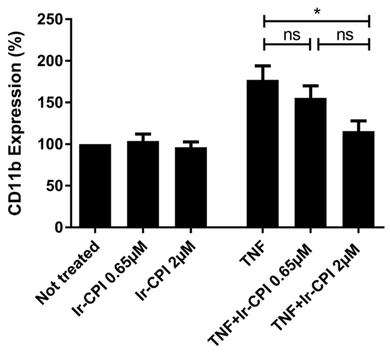
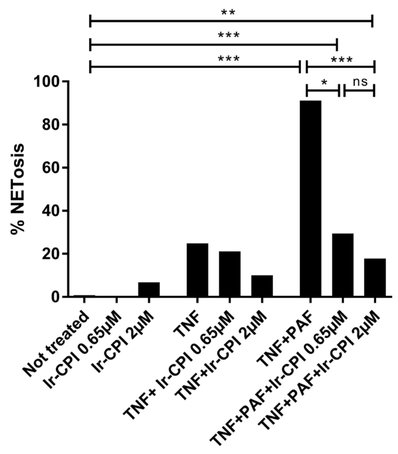


Figure 4. Analysis of CD11b expression at the surface of neutrophils. 0.65 µM and 2 µM corresponds to 5.0 µg/mL and 15.3 µg/mL of Ir-CPI, respectively. TNFa was used at 2 ng/mL. Data are presented as mean ± SEM (n=3 independent experiments). A one-way ANOVA followed by a Tukey's multiple comparison test were used for statistical analysis (ns: not significant;*p<0.05).

E. Ir-CPI prevents NETosis *in vitro*

Figure 5a. NETosis assessment under different experimental conditions. Percentage of NETosis (median) was calculated after analyzing of immunofluorescent staining and NETs length (n=5 independent experiments; 5 random fields per condition were analyzed). 0.65 µM and 2 µM corresponds to 5.0 µg/mL and 15.3 µg/mL of Ir-CPI, respectively. TNFa and PAF were used at 2 ng/mL and 25 µM, respectively. A Wilcoxon test was used for statistical analysis (ns: not significant; *p<0.05; **p<0.01; ***p<0.001).



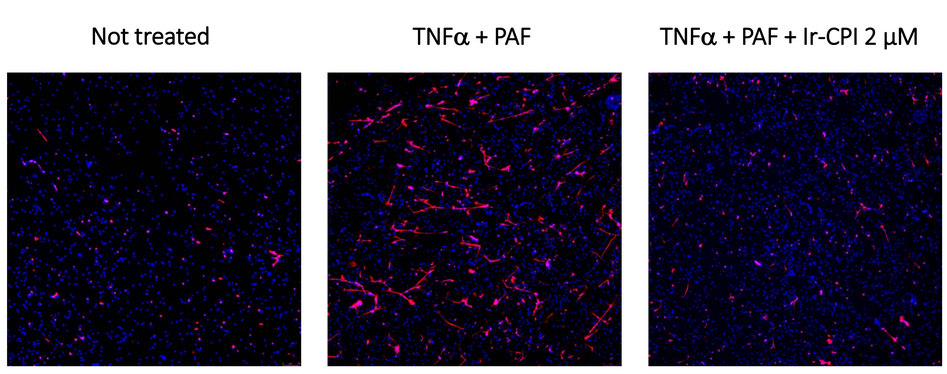


Figure 5b. Immunofluorescent visualization of NETs by staining of DNA (Hoechst) and histones (H3Cit). NETs are defined as fibers of 50 µm.

CONCLUSIONS

- Ir-CPI demonstrates an efficient **antithrombotic effect** in the **DVT model** (FXII-dependent).
- Ir-CPI is also **unexpectedly active** in the **laser injury model** (TF-dependent).
  - ✓ Ir-CPI inhibits thrombus formation, accumulation of platelets but also the **early appearance of neutrophils**. Ir-CPI **inhibits cancer-associated thrombosis** in an ectopic pancreatic cancer model.
- Ir-CPI **alters neutrophil activation** (CD11b expression) and **prevents NETs** formation *in vitro*.
  - ➔ The results suggest that Ir-CPI has **additional targets** than FXIa and FXIIa and offers **new therapeutic opportunities** in **thrombosis-associated diseases** such as cancer.

REFERENCES

[1] **Decrem Y et al.** Ir-CPI, a coagulation contact phase inhibitor from the tick Ixodes ricinus, inhibits thrombus formation without impairing hemostasis. *J Exp Med 2009; 11; 2381-2395.*

[2] **von Brühl M et al**. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med 2012; 4; 819-835.*

[3] **Darbousset R et al.** Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. *Blood 2012; 10; 2133-2143.*

[4] **Mezouar S et al.** Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo. *Int J Cancer 2015; 136; 462-475.*

[5] **Dubois C et al.** Thrombin-initiated platelet activation in vivo is vWF independent during thrombus formation in a laser injury model. *J Clin Invest 2007; 4; 953-960.*