Title

Crizotinib promotes the occurrence of inferior vena cava thrombosis and pulmonary embolism in cold-exposed mice by affecting endothelial cells and macrophages

Journal Name

Molecular and Cellular Biochemistry

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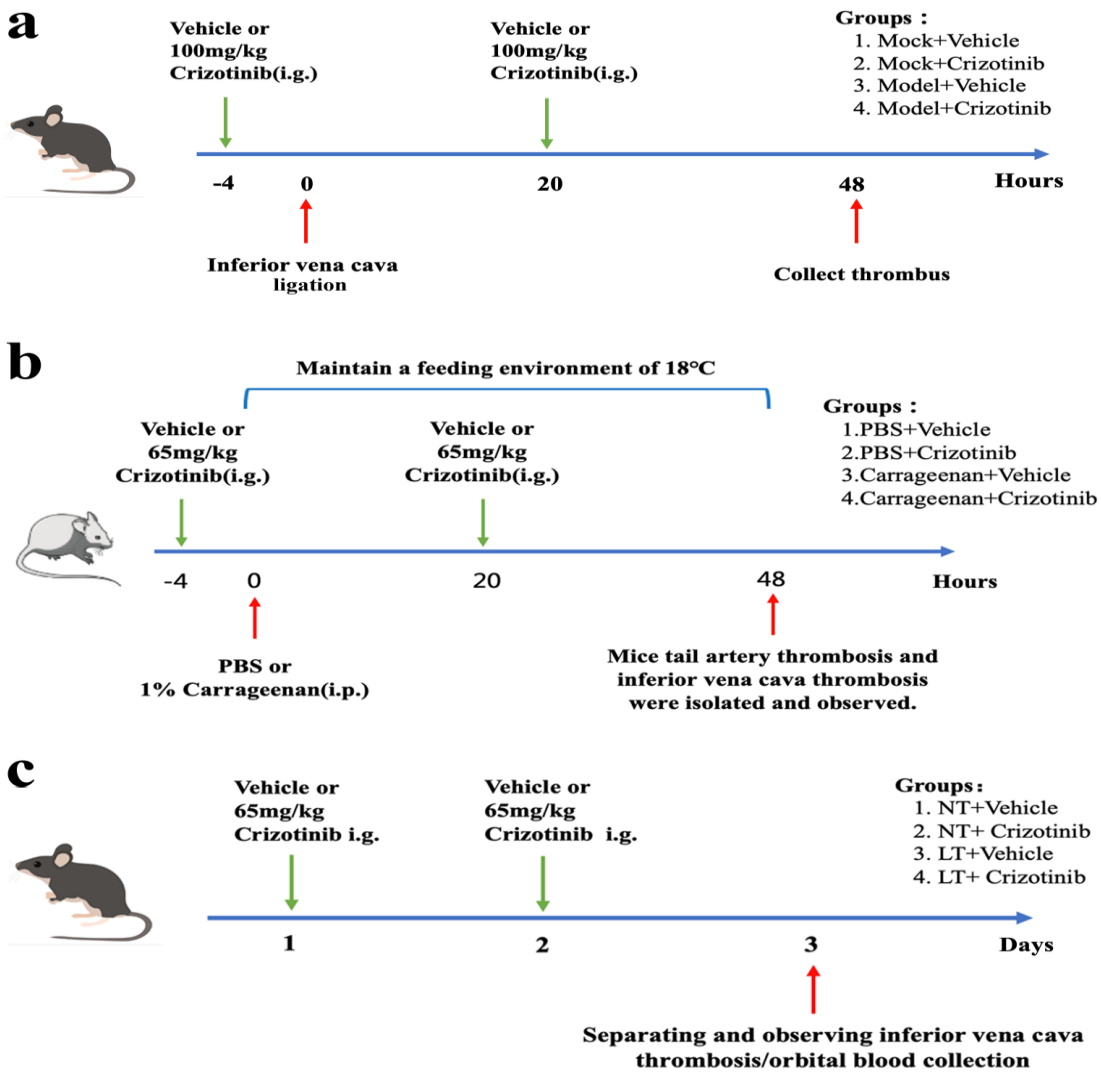
Supplementary table 1

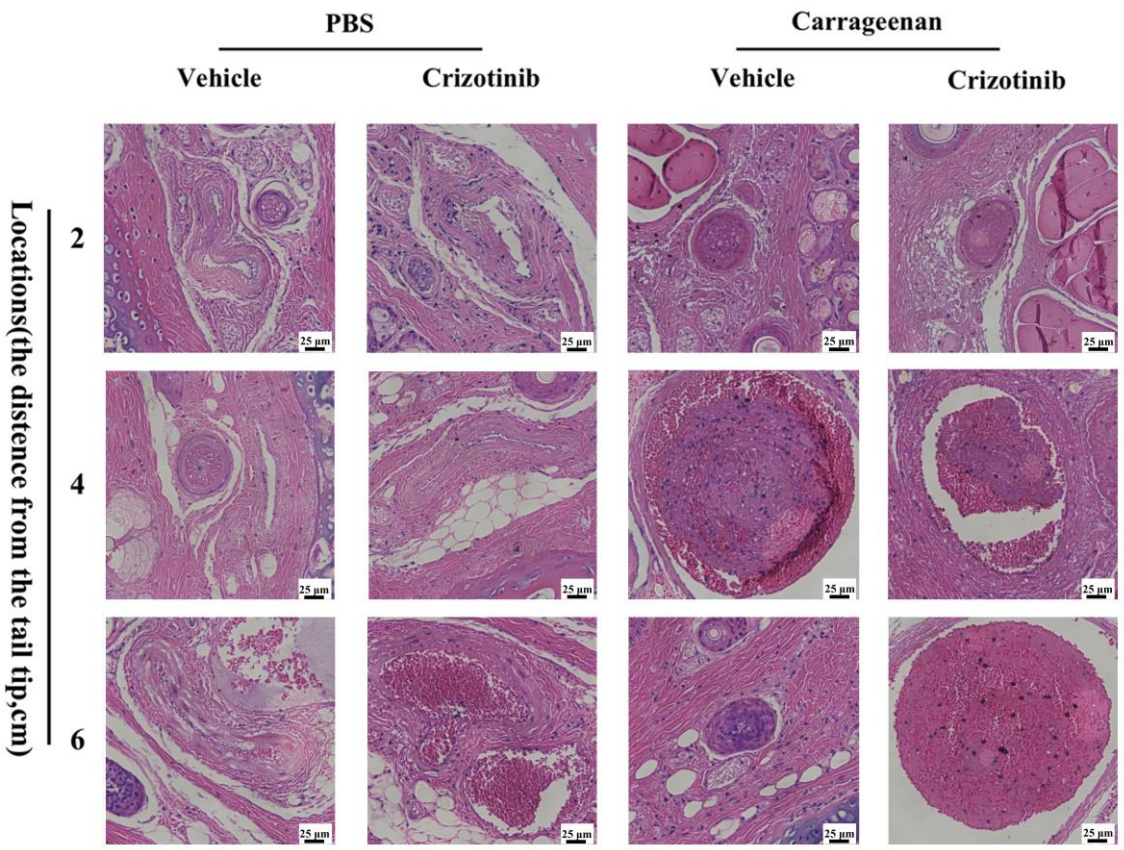
Crizotinib induces mortality in C57BL/6J mice that have undergone inferior vena cava ligation surgery

Group	Mock+Vehicle	Mock+crizotinib	Model+Vehicle	Model+crizotinib
Mice Number	8	8	8	8
Death Number	0	0	0	2
Mortality Rate	0	0	0	25%

Inferior vena cava ligation surgery is performed on the model group of mice, while a sham laparotomy is performed on the mock group. They are then administered a dose of 100mg/kg crizotinib or an equivalent volume of PBS via oral gavage, followed by close observation.

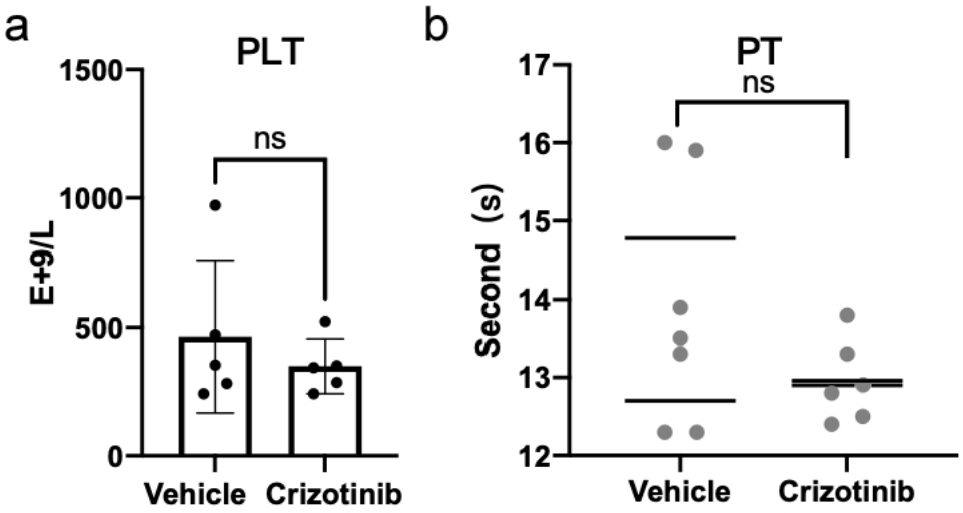


Supplementary Figure 1　Experimental Procedures Crizotinib (65mg/kg) was administered via oral gavage, with a second dose given 24 hours later. Close observation was maintained, and on the third day, mice were euthanized and thrombus-related tissue samples were collected for subsequent experiments. (a) The inferior vena cava ligation surgery model in C57BL/6J mice and the administration process of crizotinib (results shown in Figure 1). Surgery was performed on the model group of mice 4 hours after the first dose of crizotinib, while a sham surgery was performed on the control group. (b) The carrageenan-related thrombosis model in Balb/c mice and the administration process of crizotinib (results shown in Figure 2). Four hours after the first dose of crizotinib, 200μL of 1% carrageenan was injected intraperitoneally into the model group of mice, while 200μL of PBS was injected into the control group. (c) The effect of crizotinib on thrombosis was studied in C57BL/6J mice (LT) (kept under cold exposure conditions 18℃). The administration method was the same as above, with normothermic mice (NT) (kept at room temperature, 25℃) of the same strain used as controls. VT: Venous Thrombosis; Mock: Laparotomy Sham Surgery; Model: Inferior Vena Cava Ligation Surgery Modeling; Vehicle: Control for Crizotinib, i.e., DMSO; PBS: Control for Carrageenan; i.g.: intragastric; i.p.: Intraperitoneal; NT: Normothermic (maintained at room temperature, 25℃); LT: Lower than Ambient Temperature (maintained at 18℃).



Supplementary Figure2 Crizotinib Exacerbates Tail Thrombosis in Carrageenan-Induced Models

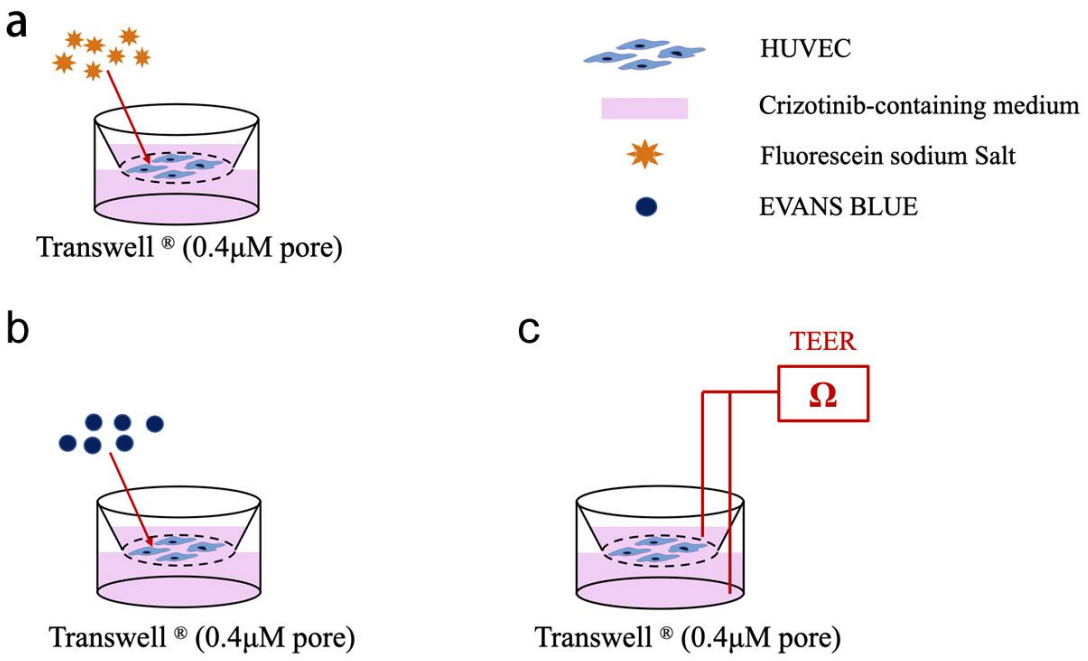
In our study investigating carrageenan-induced tail thrombosis, we confirmed that Crizotinib worsens thrombus formation in mouse tails. After 48 hours of 1% carrageenan injection, we harvested tail tissues and performed cross-sections at different distances from the tail tip (2 cm, 4 cm, and 6 cm). Subsequent hematoxylin and eosin (H&E) staining revealed the location of the tail vessels in each group, as indicated by the blue-boxed regions.



Supplementary Figure 3 Crizotinib Does Not Affect Platelet-Related Coagulation Function

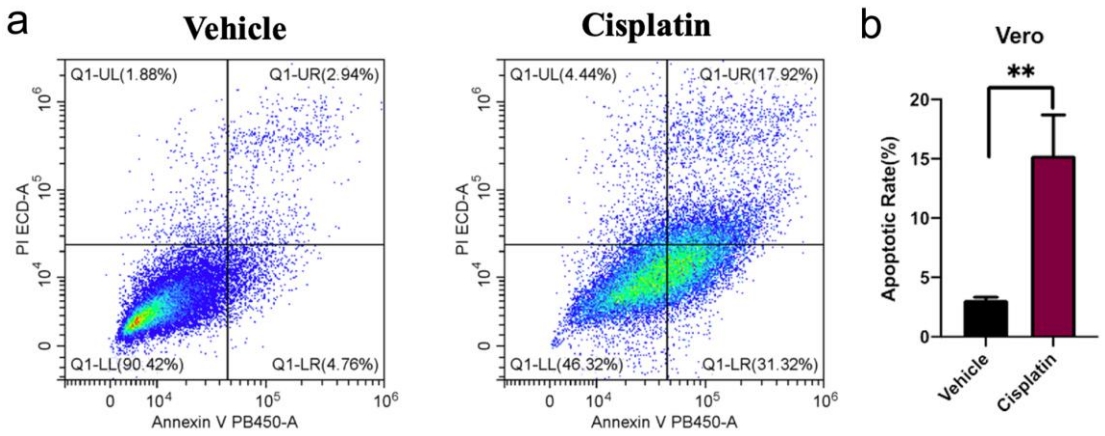
(a) After 2 days of treatment with 100 mg/kg Crizotinib, whole blood was collected for platelet counting.

(b) After 2 days of treatment with 65 mg/kg Crizotinib, blood was collected for prothrombin time (PT) measurement.



Supplementary Figure 4 Assessment of Crizotinib's Impact on Monolayer Endothelial Cell Permeability

(a) Measurement of small-molecule fluorescein sodium permeability. (b) Measurement of large-molecule Evans blue permeability. (c) Measurement of transendothelial electrical resistance (TEER) in monolayer endothelial cells.



Supplementary Figure 5 Apoptosis Induction in Vero Cells by Cisplatin

(a) Vero cells were treated with 10 μM cisplatin or PBS for 48 hours. Apoptosis was assessed using Annexin V/PI double staining and flow cytometry.

(b) Statistical analysis of the percentage of late-stage apoptotic cells.