Pre-administration of a carboxypeptidase inhibitor enhances plasminogen accumulation and thrombolysis after tPA infusion: Demonstration by real-time intravital imaging analysis of microthrombi in mice

博士（光医工学）　Nitty Skariah Mathews

論文題目

Pre-administration of a carboxypeptidase inhibitor enhances plasminogen accumulation and thrombolysis after tPA infusion: Demonstration by real-time intravital imaging analysis of microthrombi in mice

論文の内容の要旨

Introduction:

In acute cardiovascular conditions like myocardial infarction, stroke, and pulmonary embolism, resilient thrombi occlude important blood vessels, and timely treatment with thrombolytic agents like recombinant tissue-type plasminogen activators (rt-PA) is warranted. These agents activate the proenzyme Glu-plasminogen (Glu-plg) to plasmin through a conformational change by binding its lysine-binding sites (LBS) to the carboxy-terminal lysine (CTL) residues on the surface of fibrin and facilitate fibrinolysis. However, several studies have established that this group of drugs has complications like bleeding, including life-threatening intracranial hemorrhage and risk of re-occlusion.

The confluence of tissue-type plasminogen activator (tPA) and its substrate Glu-plg on the surface of the fibrin thrombi forms a complex that potentiates plasmin generation and facilitates fibrinolysis. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a plasma procarboxypeptidase that regulates the fibrinolytic process. On activation to TAFIa (activated TAFI) by thrombin-bound thrombomodulin, it cleaves the CTLs from partially degraded fibrin, thus reducing the number of CTLs for tPA-Glu-plg complex to bind, and inhibits fibrinolysis. Inhibitors to TAFI are currently being investigated for their use in thrombolytic treatment due to the shortcomings of current day thrombolytics.

In a real-time intravital microthrombus imaging model, our lab had previously established the spatiotemporal organization of the thrombus and reported that platelets get activated and express phosphatidylserine on their surface at the core of the thrombi, and Glu-plg accumulates after fibrin formation at the same region in an LBS-dependent and plasmin activity-dependent manner. Here, we use a similar model to analyze the functional role of TAFIa and Glu-plg accumulation in vivo using labeled Glu-plg and a carboxypeptidase inhibitor from potato tuber (PTCI) in Green Fluorescent Protein (GFP)-expressing mice. Subsequently, we scrutinize the process of thrombolysis using rt-PA and authenticate a potential role for PTCI as an adjunct fibrinolytic agent.

Materials & Methods:

The experiments were approved by the Animal Experiments Committee of Hamamatsu University School of Medicine (Permit number: 2020046). In conjunction with real-time intravital two-photon excitation fluorescence microscopy (Nikon A1RMP), we produced and imaged laser-induced microthrombi in the mesenteric venules of GFP mice and examined microthrombus dynamics and thrombolysis patterns in vivo by measuring the changes in the fluorescence intensity of Glu-plg labeled with Alexa Fluor 555 (Glu-plg-AF555) following administration of epsilon aminocaproic acid (EACA, a synthetic lysine analogue), PTCI, and rt-PA. The integrated NIS-Elements software was used to operate the microscope and process raw data. ImageJ software was used to analyze data. Descriptive statistics were presented as mean with standard deviations of relative fluorescence intensities (RFI) of Glu-plg-AF555 and GFP. Kruskal Wallis test was used to compare the difference across all groups, followed by a post hoc pair-wise comparison using Mann Whitney U test. P-value < 0.05 was considered as statistically significant.

Results:

PTCI significantly enhanced Glu-plg accumulation at the core of the thrombus by inhibiting TAFIa, while EACA inhibited this process ($P = 0.002$). Exogenous rt-PA effectively triggered Glu-plg activation within the thrombus and promoted thrombolysis. Administration of PTCI and rt-PA together showed no significant benefit on thrombolysis compared to rt-PA administration alone as evidenced by indifference in the time to achieve peak Glu-plg-AF555 RFI and time to near-complete clot lysis between the two groups ($P = 0.690$ and $P = 0.151$, respectively). However, early-phase systemic administration of PTCI before thrombolytic therapy by rt-PA expedited clot lysis as evidenced by significantly faster time to reach peak Glu-plg fluorescence intensity and shorter time to achieve near-complete clot lysis ($P = 0.014$ and $P = 0.003$, respectively).

Discussion and Conclusions:

Using real-time intravital imaging analysis of laser-induced mesenteric vein thrombus in mice, we aimed to establish the physiological relevance of TAFI in the regulation of fibrinolysis. Along with previous data that phosphatidylserine-exposing platelets and fibrin formation occur at the core of the thrombus, we highlight the importance of this site and encourage the development of novel fibrinolytic agents targeting this region. We confirmed that the TAFIa inhibitor PTCI enhanced Glu-plg accumulation at the thrombus core and concluded that adequate systemic inhibition of TAFIa before thrombolytic therapy by rt-PA is relevant to obtain effective enhancement of thrombolysis.

The findings of our study are in keeping with the results of a few previous studies in murine models that showed PTCI enhances the thrombolytic potential of tPA. However, it is not clear whether PTCI can induce clot lysis without exogenous tPA as there are contradictory data on this front. In this study, PTCI did not induce thrombolysis without exogenous rt-PA. Further, pre-administration rather than co-administration of PTCI with rt-PA showed a distinct benefit, with faster lysis time and earlier near-complete thrombolysis.

Based on our findings and understanding of the spatiotemporal regulatory mechanism of thrombolysis, we reiterate that PTCI, and TAFI inhibitors in general, show great promise in managing thrombotic diseases. More studies are needed to assess the pharmacokinetic and pharmacodynamic profile of this group of drugs for potential use in thrombolysis or thromboprophylaxis.