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REGULAR ARTICLE

A segment of *Staphylococcus aureus* clumping factor A with fibrinogen-binding activity ($ClfA_{221-550}$) inhibits platelet-plug formation in mice

Chao-Zong Liu [a,*], Tur-Fu Huang [b], Po-Jun Tsai [c], Pei-Jane Tsai [d], Ling-Ya Chang [a], Mei-Chi Chang [e]

[a] *Department of Pharmacology, College of Medicine, Tzu Chi University, 701, Chung-Yang Road, Sec. 3, Hualien 970, Taiwan*
[b] *Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan*
[c] *Department of Pharmacy, Dalin Tzu Chi Buddhist General Hospital, Dalin, Chia-Yi County, Taiwan*
[d] *Department of Laboratory Medicine and Biotechnology, College of Medicine, Tzu Chi University, Hualien, Taiwan*
[e] *Biomedical Science Team, Chen Kang College of Technology, Kwei Shan, Tao-Yuan County, Taiwan*

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Abstract We previously reported that the fibrinogen-binding segment (residues 221–550) of *Staphylococcus aureus* clumping factor A (ClfA), which binds to fibrinogen γ chain C-terminus, exerted inhibitory effects on platelet aggregation and fibrin clot formation in vitro. Here, we further demonstrated the effectiveness of using $ClfA_{221-550}$ to inhibit platelet-rich thrombus formation in vivo. Platelet-rich thrombi were formed in the mesenteric venules of fluorescein-loaded mice by filtered light illumination. It grew rapidly and ultimately resulted in the cessation of blood flow due to vessel occlusion. Given by intravenous bolus injection, $ClfA_{221-550}$ delayed occlusive thrombi formation in a dose-dependent manner: 2-, 3- and 4.5-fold prolongations of vessel occlusion time were attained with 0.69, 6.9 and 34.5 mg/kg of $ClfA_{221-550}$, respectively. Reduced fibrin clot formation at the late phase with plasmas, which were prepared from $ClfA_{221-550}$-treated mice, was also dose-dependent. The suppression of fibrin formation ex vivo coincided with the delay of occlusive thrombus formation in vivo, suggesting that the antithrombotic effect of $ClfA_{221-550}$ may result from the blockade of fibrinogen γ chain C-terminal functions, in mediating platelet aggregation and fibrin clot formation. Administration of $ClfA_{221-550}$ also lengthened the tail bleeding of mice; however, significant effect was achieved only with a higher dosage, namely 34.5 mg/kg. These results together

Abbreviations: ClfA, clumping factor A; GP, glycoprotein; GST, glutathione *S*-transferase; TBS, Tris-buffered saline; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; aPTT, activated partial thromboplastin time; PT, prothrombin time.

* Corresponding author. Tel.: +886 3 856 5301x7511; fax: +886 3 856 1465.
E-mail address: czliu33@mail.tcu.edu.tw (C.-Z. Liu).

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showed that blockade of fibrinogen γ chain C-terminus with $ClfA_{221-550}$ preferentially affected platelet-rich thrombus formation rather than normal haemostasis, thus providing a rationale for selecting fibrinogen γ chain C-terminus as a new target for thrombotic intervention.

Introduction

Fibrinogen is a large plasma protein (~340 kDa) that consists of two sets of three different polypeptide chains termed Aα, Bβ and γ, which, linked by disulfide bridges, form a symmetric structure with a central E domain and two outer D domains [1]. Fibrinogen mediates platelet adhesion and aggregation via binding to platelet glycoprotein (GP) IIb/IIIa [2,3] and forms complex fibrin clot in response to thrombin [4,5] and coagulation factor XIII [6,7], thus playing an important role both in thrombosis and haemostasis. Elevated plasma fibrinogen level has been described as a risk factor of venous and arterial thrombosis [8–10], whereas patients with fibrinogen deficiency suffer bleeding disorders [11,12].

It is recognized that the fibrinogen γ chain C-terminus locating at the outer D domain mediates important fibrinogen functions as it contains the platelet GPIIb/IIIa recognition sequence ($QAGDV^{411}$) [13], factor XIIIa cross-linking sites (Gln 398 and Lys 406) [14], and sites for fibrin monomer polymerization and lateral association [15]. Recently, it was reported that the hamster monoclonal antibody 7E9, which binds to the γ chain C-terminus of fibrinogen, exerts inhibitory effects on platelet aggregation, clot retraction and fibrin clot formation [16,17], and mice treated with 7E9 developed only small, non-occlusive thrombi in injured carotid artery [18]. According to these results, blockade of fibrinogen γ chain C-terminus is considered as a new antithrombotic strategy [19].

Staphylococcus aureus (*S. aureus*) bacterium is one of the major causes of both nosocomial and community acquired infections. This pathogen produces a family of cell wall-anchored surface proteins which enable organism to adhere to extracellular matrix component of the host for colonization [20]. Clumping factor A (ClfA) is the prototype of this family. It binds with a high affinity to fibrinogen γ chain C-terminus thus mediating *S. aureus* clumping in the presence of fibrinogen [21,22]. The fibrinogen-binding activity of ClfA has been localized to the residues 221–550 ($ClfA_{221-550}$) [23], which folds into two immunoglobulin-like domains and presents a binding pocket at domains' interface for fibrinogen γ chain C-terminal peptide QAGDV to dock into [24]. From these data, we envision that the fibrinogen-binding segment of ClfA ($ClfA_{221-550}$) may be a potential candidate for blocking the function of fibrinogen γ chain C-terminus, in mediating platelet aggregation and fibrin cross-linking, thereby inhibiting thrombus formation. This idea was strongly supported with our previous observations that recombinant $ClfA_{221-550}$ inhibited human platelet aggregation, fibrin clot formation and platelet-mediated clot retraction through binding to fibrinogen [25]. In this study, the antithrombotic activity of $ClfA_{221-550}$ was further demonstrated with its capacity to inhibit platelet-rich thrombi formation in mesenteric venules of mice. More importantly, a significant antithrombotic effect could be achieved with $ClfA_{221-550}$ at a dosage that did not affect normal haemostasis in terms of arresting tail bleeding. These results reinforce the previous conclusion that blockade of fibrinogen γ chain C-terminus is a novel antithrombotic approach.

Materials and methods

Materials

Glutathione Sepharose 4B gel and bovine thrombin were purchased from Amersham Biosciences Ltd (Uppsala, Sweden). Glutathione (reduced form), BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/4-nitroblue tetrazolium chloride) solution and fluorescein sodium were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium pentobarbital (Somnotol®) and aspirin (Aspefic®) were commercial products of MIC Pharmaceuticals (Ontario, Canada) and Laboratoies Synthelabo-Synthelabo Goupe (Quetigny, France), respectively. ICR mice (male) were provided by the animal center of National Taiwan University Hospital (Taipei, Taiwan) and this animal study was approved by the Institutional Committee on the Use and Care of animals of Tzu Chi University. All the mice were anesthetized by intra-peritoneal injection of sodium pentobarbital (50 mg/kg) prior to carry out experiments.