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Statin Therapy before Percutaneous Coronary Intervention: A Novel Bridge between Thrombin and Thrombomodulin for Enhanced Cardiovascular Protection

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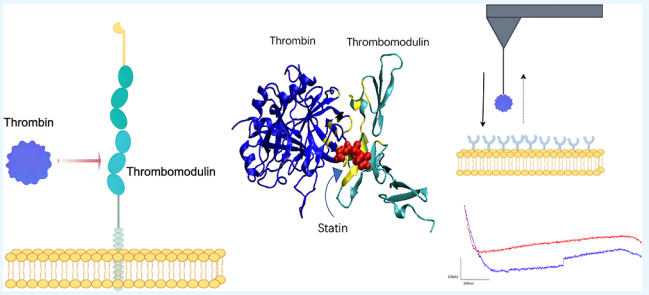
ABSTRACT: Percutaneous coronary intervention administration of statins enhances the thrombin−thrombomodulin (TM) interaction, which is critical for the prevention of post-procedural cardiovascular complications. Atomic force microscopy showed that statins increased the single molecule rupture forces between thrombin and TM, suggesting a strengthened complex. This was supported by surface plasmon resonance, which showed an enhanced binding affinity under the statin treatment. Molecular docking and dynamics simulations revealed that statins can bind to both thrombin and TM, forming stable complexes that facilitate protein C activation. Clinically relevant doses of statins were found to significantly enhance the thrombin−TM interaction, potentially reducing the risk of myocardial infarction and stent thrombosis by augmenting the endothelial anticoagulant properties through the thrombin−TM pathway. By acting as a bridge, statins foster more stable thrombin−TM complexes, supporting endogenous anticoagulant mechanisms and leading to better cardiovascular outcomes.



1. INTRODUCTION

Statins effectively lower plasma lipid levels by inhibiting the key rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which is crucial for reducing the risk of cardiovascular diseases. Given this mechanism of action, statins have gained increasing recognition in preventive medicine and are widely used for both primary and secondary prevention of cardiovascular diseases.

Particularly in the treatment of acute coronary syndromes (ACS), the latest clinical guidelines recommend the early use of statins, if possible, prior to percutaneous coronary intervention (PCI).[1] This recommendation is based on the results of numerous studies and meta-analyses, which have shown that the use of statins before PCI, as opposed to after, significantly reduces the incidence of myocardial infarction (MI), thereby decreasing the risk of major adverse cardiac events (MACE). Meta-regression analyses further confirm that early administration of statins is closely associated with a reduction in the risk of MI and MACE, and this benefit persists in long-term follow-up.[2−4]

However, the detailed mechanisms of this phenomenon are still unclear. The short duration of statin therapy may not be sufficient to exert its lipid-lowering effects, thereby failing to provide clinical benefits, while the study of statin's pleiotropic mechanisms is expected to contribute to the clinical outcomes of early statin treatment. Recent studies have highlighted their anticoagulant effects, which are primarily achieved by reducing the production of tissue factor (TF) and upregulating the expression of thrombomodulin (TM) on endothelial cell. This results in a reduction in thrombin generation, which subsequently diminishes the incidence of various thrombin-catalyzed procoagulant reactions.[5] Previous experimental studies have also indicated that statins could inhibit platelet function,[6] but the intensity of antiplatelet therapy was already adequate for most patient with ACS because of the dual antiplatelet therapy.

Thrombin, one of the potent stimulators of platelets and the end product of the coagulation system, is responsible for converting fibrinogen into fibrin to stabilize the platelet plugs. Thrombin has a strong affinity for TM on the endothelial membrane, and this critical interaction significantly promotes the production of activated protein C (aPC), which is a key factor in inhibiting the clotting cascade.[7] In addition to the expression level of TM, the affinity between thrombin and TM also significantly affects the formation of thrombin−TM complexes, which has never been studied before.

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Preliminary findings indicate that rosuvastatin, at therapeutic doses, can enhance the interaction between thrombin and TM. However, there are currently no studies exploring whether statins influence the formation of thrombin−TM complexes. Given the anticoagulant properties of statins, we hypothesize that various statins affect the kinetics of thrombin−TM interactions, thereby activating the major natural anticoagulant protein C system. This study aims to explore the mechanisms by which earlier statin administration can reduce the incidence of MI following PCI. Therefore, we investigated the effects of three statins: rosuvastatin, atorvastatin, and pitavastatin on the thrombin−TM interaction and their potential mechanism.

2. RESULTS

2.1. Statins Significantly Increase the Affinity of Thrombin for TM and the Rate of Protein C Activation. Determination of the binding affinity for thrombin−TM was inferred from protein C activation experiments, yielding equilibrium dissociation constant (K_d) values ranging from 186 to 225 nmol/L. The introduction of various statins led to reduced K_d values, suggesting heightened affinity for TM. Notably, as the concentrations of statins were escalated, the TM affinity consistently increased. However, it is noteworthy that the maximum rate of protein C cleavage (V_{max}) remained unchanged across different statin concentrations; refer to Figure 1 for a detailed illustration.

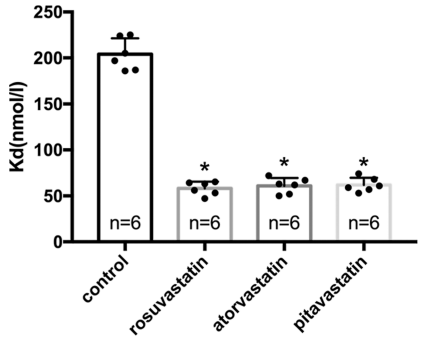

Figure 1. Effect of statins on the K_d values of thrombin−TM interaction; the K_d value of three kinds of statin groups was significantly lower than that of the control group. *$P < 0.05$ for Student's t test, n represents number of duplicate wells. Further investigation justifies the exclusion of statins' influence on protein C activation by free thrombin. Experimental data indicate that in the absence of TM, all statins tested did not affect protein C activation, even at concentrations as high as 10^{-5} mol/L.

2.2. Assay for Thrombin Using TM-Coated Microplates. In an effort to substantiate the influence of statins on the thrombin−TM interaction, binding assays were conducted using TM-coated microplates. The presence of various statins was observed to significantly enhance the affinity of thrombin for TM. In particular, a dose-dependent reduction in K_d was observed, indicating an enhanced binding affinity at therapeutic concentrations of statins. In the absence of statins, the K_d values were found to be consistent with those obtained from the protein C activation assays. A direct correlation between statin concentrations and enhanced thrombin affinity for TM is observed, as illustrated in Figure 2.

2.3. Statins Increased the Binding Force of Thrombin/TM. Given that native TM is present on the COS-7 cell surface, we employed atomic force microscopy (AFM) to investigate the biological impact of statins on the thrombin−TM interaction by analyzing the rupture forces. The data indicated a significant augmentation in the rupture force between thrombin and TM in the presence of each of the three distinct statins tested, relative to the absence of statins, as shown in Table 1 and Figure 3.

2.4. Statins Interacted with Thrombin and TM Simultaneously. Molecular docking studies provided further insights into the interactions between statins and the thrombin−TM complex. We analyzed five distinct structural compounds for each statin category, detailed in Table S1. The computational approach yielded insights that were in accordance with the experimental findings described previously, as shown in Figures 4 and 5. The results indicated that statins interacted with both thrombin and TM simultaneously, suggesting a potential dual mode of interaction. This observation was significant in that it provided a structural basis for understanding the enhanced affinity of thrombin for TM in the presence of statins. The molecular docking studies demonstrated that statins could potentially modulate the thrombin−TM interaction, which may contribute to the observed clinical effects of statins in reducing thrombotic events. These findings are consistent with the established role of thrombin as a key enzyme in the coagulation cascade, and the ability of statins to influence this interaction could have important implications for the use of statins in the prevention and treatment of thrombotic disorders.

3. DISCUSSION

This study utilized three distinct experimental approaches to elucidate the effects of statins on the thrombin−TM interaction. These were further supplemented by molecular docking and molecular dynamics (MD) simulations, which aimed to clarify the molecular interactions and stability of the thrombin−TM complex. The comprehensive analysis conducted across all employed methods indicated that rosuvastatin, atorvastatin, and pitavastatin were observed to intensify the thrombin−TM interaction, as evidenced by the decreased K_d values, which indicate enhanced binding affinity. The observed enhancement in binding affinity may be attributed to the ability of statins to act as bridging molecules, potentially forming hydrogen bonds or hydrophobic interactions between thrombin and TM. This augmented binding affinity indicates that statins may contribute to anticoagulation benefits via a supplementary mechanism.

Statins, a diverse group of medications prevalent in medical settings, exhibit variable solubility profiles, which can be attributed to their distinct chemical structures. To illustrate, rosuvastatin is highly water-soluble, in contrast to atorvastatin and pitavastatin, which are more lipophilic. The selection of statins for this investigation was guided meticulously by their respective oil−water partition coefficients, which correlate with their solubility and consequently their bioavailability and therapeutic action. To corroborate our prior findings, we employed three experimental techniques to assess the impact of statins on thrombin−TM binding. The data indicated that all three statins, namely, rosuvastatin, atorvastatin, and pitavastatin, enhanced the interaction, thereby suggesting a potential mechanism by which these drugs increase the formation of the thrombin−TM complex, which in turn boosts the activation of protein C, a crucial anticoagulant factor. The anticoagulation system of the protein C pathway, which requires the binding of TM and thrombin to become activated, plays an important role in the process of anticoagulation. Following the binding of TM with thrombin, the activity of thrombin was significantly reduced, thereby impairing its ability to activate coagulation

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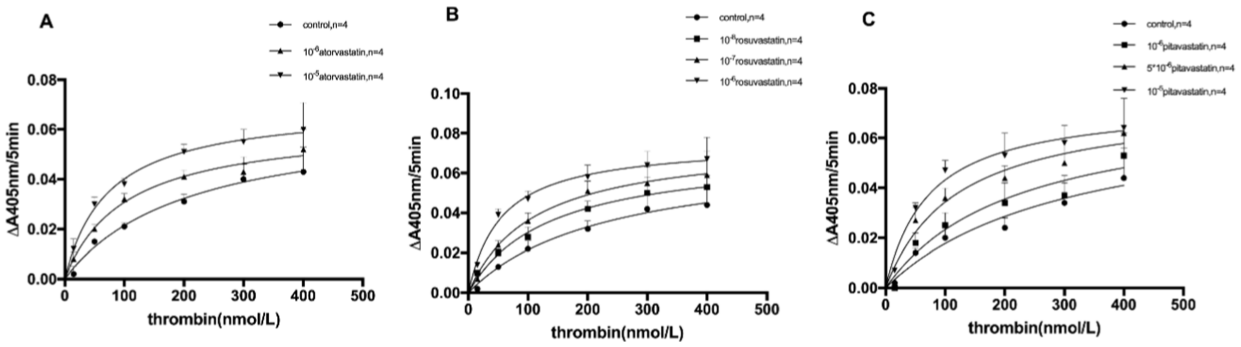

Figure 2. Effect of statin on the formation of thrombin−TM, (A) atorvastatin group, (B) rosuvastatin group, and (C) pitavastatin group. It shows a dose-dependent increase in absorbance of the solution at 405 nm in the presence of different concentrations of statin, *n* represents number of duplicate wells. Note: absorbance at 405 nm (A405 nm) was measured to quantify the binding activity of thrombin and TM in the presence of statins. This wavelength was chosen for its optimal sensitivity in detecting the color change associated with the enzymatic reaction, and the absorbance correlates directly with the product amount, reflecting binding activity.

Table 1. Effect of Statins on the Single-Molecule Interactional Force between TM and Thrombin

groups	interactional force (pN)	
	control	statin
rosuvastatin	40.71 ± 0.73	74.41 ± 4.41[a]
atorvastatin	45.87 ± 1.22	83.98 ± 3.46[a]
pitavastatin	50.04 ± 1.07	87.01 ± 2.00[a]

[a]$P < 0.05$ for Student's t test.

factors V, VIII, and platelets. Conversely, binding of TM with thrombin enhanced the rate of thrombin activation of protein C by over 1000-fold. Protein C, in turn, can inactivate factors V_a and $VIII_a$, thereby inhibiting coagulation reactions.[8]

The varying concentrations of statin in the solid-phase assay demonstrated a correlation between statin levels and the thrombin−TM binding affinity. Our analysis revealed that as the concentration of statin increased within the therapeutic range, the K_d value, which is indicative of the thrombin−TM binding affinity, decreased correspondingly. A lower K_d value denotes a higher affinity. While previous studies have explored the roles of statins in different coagulation pathways, no investigation has been conducted to determine whether different types of statins exert similar effects on the same anticoagulant pathway target. This is because different statins have different molecular structures and may have inconsistent effects on the same anticoagulant pathway. Our results indicated that the influence of statins on the thrombin−TM interaction may be a common feature of statins, representing a novel perspective in the field of anticoagulation.

The causes of coronary stent thrombosis are complex and varied. Historically, there has been a tendency to prioritize antiplatelet therapy following PCI. However, the intrinsic anticoagulation capabilities of the human body and the significance of anticoagulants have been somewhat overlooked. In patients who had experienced stent thrombosis following PCI, the thrombin generation was significantly higher compared with that in patients who had not experienced stent thrombosis following PCI. The capacity of exogenous TM to diminish thrombin generation was less pronounced in the stent thrombosis cohort. These findings indicate that the anticoagulation system of the protein C pathway maybe involved in the pathogenesis of stent thrombosis.[9] Prior research has demonstrated that aPC and its receptor are capable of releasing the injury of endothelial cells and inhibiting platelet adhesion following the implantation of an eluting stent.[10] This presents an intriguing hypothesis: statins, by enhancing thrombin−TM binding, may promote the activation of the protein C pathway, thereby improving the body's intrinsic anticoagulant response and reducing thrombus formation at the stent site. We speculate that statins' ability to enhance thrombin−TM interactions not only diminishes thrombin's procoagulant activity but also activates protein C more effectively. This pathway could play a crucial role in reducing the risk of stent thrombosis by preventing excessive thrombin generation and platelet aggregation at the stent site. In clinical practice, the additional use of rivaroxaban inhibition in patients with ACS who have received dual antiplatelet therapy after PCI has been demonstrated to reduce the risk of mortality and stent thrombosis.[11] Similarly, in patients with atrial fibrillation and PCI, nonvitamin K antagonist oral anticoagulants (NOACs) have been shown to be an effective strategy for preventing stent thrombosis. The combination of anticoagulant with a P2Y12 inhibitor proved to be an effective strategy for reducing ischemic events compared to the regimen of additional aspirin use.[1,12] These findings suggest that a broader strategy targeting the coagulation system may be beneficial in preventing both MI and stent thrombosis. Meanwhile, the animal trial demonstrated that overexpression of TM was capable of limiting femoral arteries' neointima formation and decreasing in intra-arterial thrombus formation after mechanical overdilation injury.[13] Given that animal studies have demonstrated that statins can reduce thrombus formation and neointima development by increasing TM expression and considering that the primary function of TM is to bind thrombin to exert its anticoagulant effects, we hypothesize that this mechanism is likely due to the enhanced ability of TM to capture more thrombin as a result of its increased expression. Similarly, our findings show that statins strengthen the binding affinity between thrombin and TM, which implies that more thrombin is sequestered by TM, thereby facilitating protein C activation and its subsequent anticoagulant effects. In essence, statins enhance the anticoagulant function of TM on endothelial cells across the vascular system, reducing circulating thrombin levels and promoting the activation of protein C. This effect is particularly important in stent-adjacent endothelial cells, where the anticoagulant capacity of TM is augmented by statins, reducing platelet aggregation and thrombin activity. Consequently, this mechanism likely contributes to the prevention of in-stent thrombosis.

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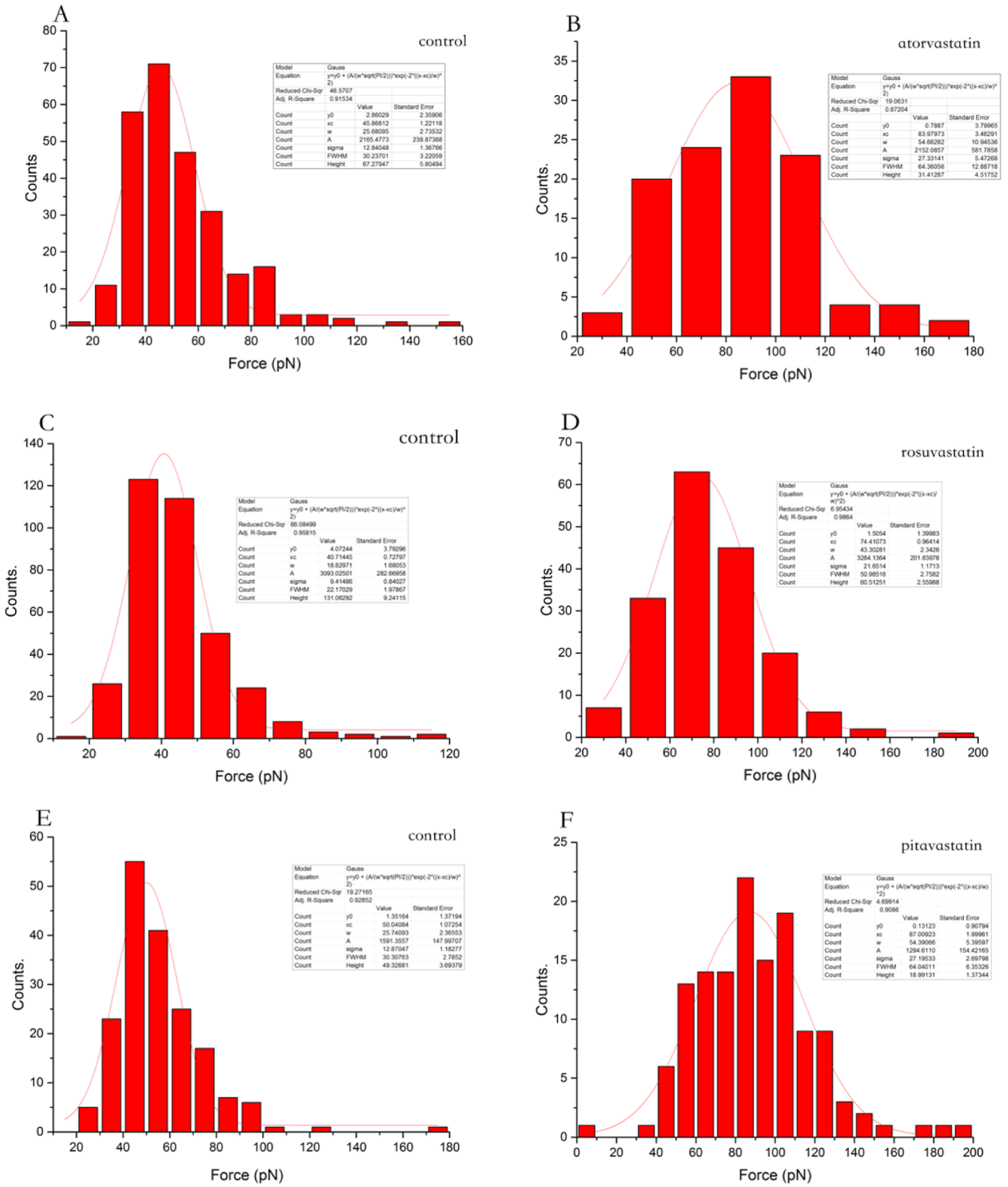

Figure 3. Effect of three kinds of statin on the single-molecule interactional force between TM and thrombin. (A) Control group, compared with atorvastatin, (B) atorvastatin group, rupture force was significantly higher (*$P < 0.05$) compared to control group; (C) control group, compared with rosuvastatin, (D) rosuvastatin group, rupture force was significantly higher (*$P < 0.05$) compared to control group; and (E) control group, compared with pitavastatin, (F) pitavastatin group, rupture force was significantly higher (*$P < 0.05$) compared to control group. *$P < 0.05$ for Student's t test.

MI after PCI is thought to be attributable to a number of factors. Primarily, acute or subacute thrombosis may occur at the site of stent implantation as a result of damage to the vascular endothelium. Second, when PCI is performed on major blood vessels, there is a potential for compression of branch vessels, leading to vascular occlusion. Third, coronary intervention may result in the activation of coagulation-promoting substances, embolization and dysfunction of micro vessels, and reperfusion

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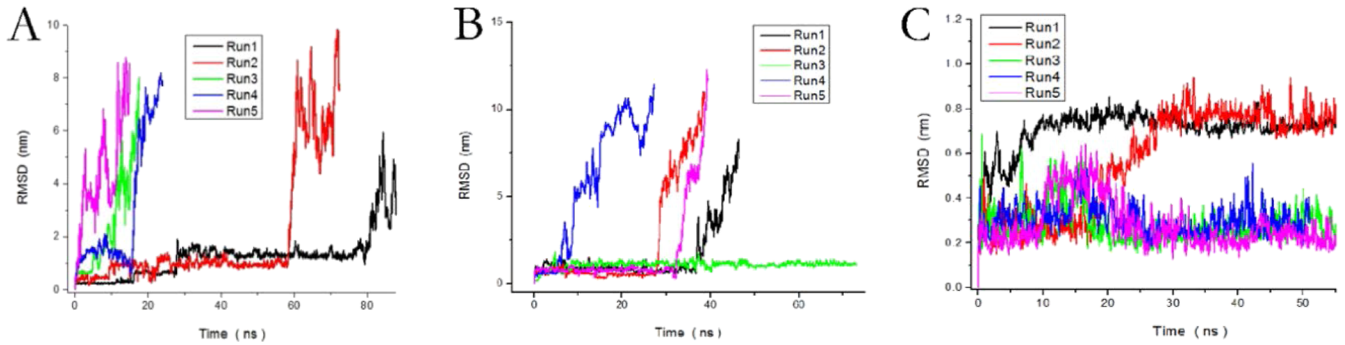

Figure 4. (A) RMSD of atorvastatin, (B) RMSD of pitavastatin, and (C) RMSD of rosuvastatin. For each statin group, Run1 to Run5, respectively, represent five possible complexes, with corresponding structures shown in Table S1.

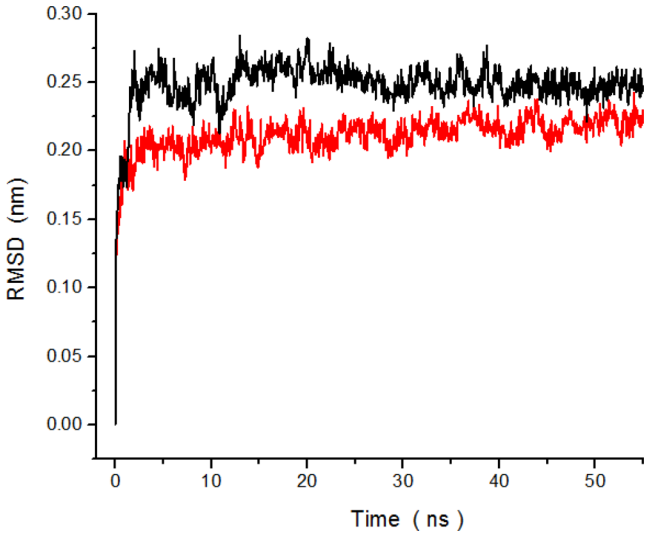

Figure 5. RMSD of rosuvastatin/thrombin/TM. The red curve represents the complex consisting of thrombin, TM, and statin, while the black is the complex without statin.

injury.[2,4,14−16] Previous research has indicated rosuvastatin treatment could lead to hemodynamic improvement for noncalcified lesions in patients with coronary artery disease. This may be attributed to the promotion of endothelial cell nitric oxide synthesis (NOS) and release, as previous studies have suggested that statins increase the phosphorylation of endothelial nitric oxide synthase.[17,18] It can be assumed that statins alter the spatial distribution of TM, thereby influencing the cytoplasmic domain of TM, which is involved in modulating the protein kinase B/NOS signaling pathway. Furthermore, the expression of TM is significantly elevated in microvascular endothelial cells.[19] The pathological effects of immunothrombosis, which occur subsequent to intravascular injury, may be regulated by the thrombin−TM interaction. This interaction activates downstream signaling pathways that modulate inflammation and coagulation within the microcirculation.[20] Therefore, statins may exert effects similar to those of increased TM expression by influencing the interaction between TM and thrombin, thereby suppressing neointima formation and thrombosis following interventional procedures. Additionally, the activation of the anticoagulant system induced by the enhanced thrombin−TM binding may mitigate the hypercoagulation state triggered by interventional therapy. Furthermore, statins alleviate vascular immune responses and the formation of immunothrombosis, both of which contribute to their ability to reduce the occurrence of MI. These mechanisms collectively underline the multifaceted role of statins in improving cardiovascular outcomes after PCI.

Despite previous studies indicating that statins reduced the production of TF and thrombin and upregulated the expression of TM,[5] these effects were unlikely to be involved in the reduction of MI and MACE. This is because the average time from oral statin administration to improvement in coronary blood flow was less than 77 min,[21] subtracting the time for drug digestion and absorption. This indicates that statins have to exert pleiotropic effects extremely fast. The AFM results indicated that the interaction among thrombin, TM, and statin was rapid. This was evidenced by the continuous approach of the cantilever bearing thrombin to the cell surface, which facilitated binding between thrombin and TM. Subsequently, the cantilever retracted from the cell surface, thereby breaking the binding between thrombin and TM. Thus, even under the influence of statins, the binding of thrombin and TM can be completed in an instant.

In light of the complex interactions between statin, thrombin, and TM, we put forth two hypotheses: (1) statin may interact with thrombin or TM independently and (2) statin may act as a "bridge", concurrently binding to both thrombin and TM. Moreover, it is possible that multiple statin molecules may engage with thrombin and/or TM simultaneously.

In order to ascertain which mechanism is responsible, molecular simulation methods were employed. The results indicated that pitavastatin and rosuvastatin could indeed serve as "bridges", thereby stabilizing the complexes. Nevertheless, the docking results did not elucidate a clear mechanism for atorvastatin's relatively high affinity in the thrombin/TM interaction. This may be due to a number of factors. First, as the molecular structure of atorvastatin is distinct from that of other statins, the mechanism by which it enhances the affinity of thrombin for TM may be unique. It would be premature to dismiss the possibility that other statin molecules may be capable of binding thrombin and TM simultaneously in light of the docking results. Second, the binding of atorvastatin to the thrombin−TM complex may result in a degree of conformational change in thrombin, TM, or atorvastatin itself, which ultimately enhances the interaction between thrombin and TM. Unfortunately, however, applying molecular docking to confirm this hypothesis is time-consuming and not widely appreciated. Finally, despite the ongoing development and improvement of molecular docking, it remains unable to provide accurate results that align with experiments.

While acknowledging the limitations of our study, these aspects highlight the necessity for further research to elucidate

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the therapeutic implications of statins in modulating thrombin−TM interactions. First, financial constraints precluded further investigation into the impact of statin concentration on thrombin−TM affinity. Second, the in vitro findings may not fully capture the complexities observed in vivo. Therefore, further studies are required to verify the effects of statins on thrombin−TM binding in the vascular endothelium, including the use of patient plasma and animal models. Third, the use of advanced techniques, such as X-ray diffraction, is essential for the elucidation of the complex's spatial conformation.

In conclusion, statins appear to facilitate thrombin−TM interactions, potentially by serving as a simultaneous link to both molecules and hence to promote the activation of the protein C system, which partially explains the use of statins before PCI can bring benefits. Further investigations are necessary to delve deeper into the mechanisms underpinning the interactions among statins, thrombin, and TM.

4. EXPERIMENTAL SECTION

Sekisui Diagnostics supplied the carboxy-terminal truncated variant of recombinant human TM, devoid of the presumptive transmembrane and cytosolic segments composed of roughly 38 amino acids. Haemtech provided human zymogen protein C. Sigma-Aldrich was the source for recombinant hirudin. Chromogenic substrates S-2366 and Phe-Pip-Arg-pNA were obtained from HYPHEN BioMed. The protein C activator derived from *Agkistrodon contortrix*, as well as statins and human alpha thrombin, was acquired from Haematologic Technologies. Thermo Scientific provided high-affinity 96-well plates (NUNC Maxisorp). PBST buffer solution (comprising 150 mmol/L NaCl, 20 mmol/L Na_2HPO_4, 20 mmol/L NaH_2PO_4, and 0.05% Tween 20 at pH 7.0) was utilized for assay development.

4.1. Experiment Based on Protein C Activation. Evaluation of the initial rate of protein C activation by the thrombin−TM complex was conducted as a function of thrombin concentration, modulated by the statin presence. This rate was instrumental in deducing the thrombin−TM interaction's apparent equilibrium dissociation constant (K_d), employing a previously established methodology.[22,23] Assays were performed in 96-well polystyrene plates, with reactions totaling 200 μL. The buffer consisted of 20 mmol/L Tris, 0.1 mol/L NaCl, 2.5 mmol/L $CaCl_2$, and 1% BSA at pH 7.50, maintained at 25 °C. Protein C (1 μmol/L) was incubated with TM (1 nmol/L) alongside a spectrum of statin types and thrombin concentrations (15−400 nmol/L). The reaction was terminated at set intervals using hirudin, and the resultant aPC levels were quantified via the S2366 chromogenic substrate (200 mmol/L).

4.2. Assay for Thrombin Using TM-Coated Microplates. The thrombin−TM complex formation was assessed through a solid-phase binding assay, employing methodologies previously documented.[23] TM at 0.25 mg/mL was immobilized onto microplate wells (100 μL/well) during an overnight incubation at 4 °C using a 50 mmol/L carbonate buffer at pH 9.50. Wells were subsequently blocked using 5% nonfat dry milk for 2 h at 37 °C. Post-blocking, wells received 150 μL of thrombin at various concentrations (15−400 nM) and were incubated for 1 h. Following incubation, wells were rinsed with PBST and then introduced to 150 μL of Phe-Pip-Arg-pNA solution (200 μmol/L), which is thrombin-specific, to quantify bound thrombin via absorbance at 405 nm using a spectrophotometer.

4.3. Atomic Force Microscopy. Recent advancements in AFM have streamlined the measurement of intermolecular forces at a molecular scale.[24] This technique involves applying a force until a molecular bond breaks or a structure unfolds, which can be detected by a force drop. Utilizing this principle, we used AFM to determine the force required to dissociate thrombin from TM. In our study, we functionalized an AFM cantilever with thrombin and presented TM on a flat glass-bound cell surface. We selected the COS-7 cell line due to its minimal TM expression, which was enhanced by transfecting pTM-GFP recombinant plasmids, allowing for sufficient TM expression. The interaction between thrombin and TM was facilitated by bringing the modified cantilever into contact with the cell surface. The cantilever, bearing thrombin, was then retracted at a consistent speed, and the force of separation was recorded using an Agilent 5500 AFM/SPM system.

The experiment was conducted under the following conditions: Group 1 comprised nontransfected cells that were not subjected to statin treatment. Group 2 included cells transfected with pTM-GFP recombinant plasmids yet not treated with statins. In Group 3, cells transfected with the plasmid were exposed to rosuvastatin (10^{-6} M) for a minimum of 1 h preceding the AFM force measurement. Similarly, Groups 4 and 5 consisted of plasmid-transfected cells incubated with atorvastatin (10^{-6} M) and pitavastatin (10^{-6} M), respectively, each for at least 1 h before force determination with AFM.

4.4. Molecular Docking and MD Simulations. We hypothesized that statins affect the binding affinity of thrombin−TM either by interacting with thrombin and TM simultaneously or by interacting with one of them. Molecular docking was used to address this issue. The crystal structure of the thrombin−TM complex was obtained from the RCSB Protein Data Bank (PDB code: 1DX5). The Autodock4.2 software package was used to predict potential binding positions of statins on both thrombin and TM. The stability of the putative binding structures of the ligand on the protein was further investigated by MD simulations. Upon completion of the docking processes, the minimum energy structure was selected as the initial structure for the MD simulations. All simulations were conducted using Gromacs 4.5.[25] The AMBER force field ff99SB was applied to the protein and the general amber force field (GAFF) was used for ligand.[26] The partial charges of the ligand were obtained from a restrained electrostatic potential (RESP) calculation. The TIP3P water model was used in this work. The Na and Cl ions were added to neutralize the system and yield the ion concentration of 0.15 mol/L. All MD simulations were performed in the *NPT* ensemble at 1 bar and 300 K; the pressure and temperature of the system were maintained using the Parrinello−Rahman barostat and the velocity-rescaling thermostat, respectively.[27] The particle-mesh Ewald (PME) method was employed to account for long-range electrostatic interactions, whereas a typical 12 Å cutoff distance was applied to calculations of short-range electrostatic and van der Waals energies. Periodic boundary conditions were applied, and the integration time step is 2 fs.[28] The root-mean-square deviation (RMSD) of the statin position was used to indicate the binding stability of the statin−thrombin/TM complexes.

4.5. Statistics. Data are presented as mean ± standard deviation. Statistical significance of the mean differences was assessed using a one-tailed Student's t test ($p < 0.05$). For multiple treatment comparisons to a control, Dunnett's test was applied.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c10348.

Conformation details of the possible conformation among statins, thrombin (left blue sphere), and TM (right Y-shaped), with statin in between in each molecular model (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS AND ACRONYMS

aPC	activated protein C
AFM	atomic force microscope
ACS	acute coronary syndromes
GAFF	general amber force field
K_d	equilibrium dissociation constant
MD	molecular dynamic
MI	myocardial infarction
MACE	major adverse cardiac event
NOS	nitric oxide synthesis
NOACs	nonvitamin K antagonist oral anticoagulants
PCI	percutaneous coronary intervention
PME	particle-mesh Ewald
RMSD	root-mean-square deviation
RESP	restrained electrostatic potential
TM	thrombomodulin
TF	tissue factor

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