CLINICAL TRIAL

Ticagrelor versus clopidogrel in reducing inflammatory cell infiltration of thrombus aspirated in patients with ST-elevation myocardial infarction

Jing Shen[1] · Guangzhong Liu[1] · Guodong Wu[2] · Lijian Sheng[2] · Zhengyu Cao[2] · Shuyuan Guo[2] · Shaohong Dong[1] · Xin Sun[1] ⬤ · Ye Tian[2]

Abstract

Background Ticagrelor provides more rapid, potent, and consistent anti-platelet efficacy than clopidogrel. This randomized trial aimed to evaluate the anti-inflammation effects of ticagrelor versus clopidogrel on thrombus aspirated from the ST-elevation myocardial infarction (STEMI) patients.

Method A total of 98 patients with STEMI and intended percutaneous coronary intervention (PCI) were randomly assigned to receive clopidogrel (600-mg loading dose) or ticagrelor (180-mg loading dose), of whom 55 with large thrombus burden underwent thrombus aspiration during PCI. Thrombus specimens were successfully aspirated from 49 patients. Finally, 24 patients in the clopidogrel group and 23 in the ticagrelor group completed the study. Inflammatory cells within thrombi were assessed by hematoxylin–eosin and immunohistochemistry stainings.

Results Compared with the clopidogrel group, the number of total inflammatory cells per mm^2 thrombus area in the ticagrelor group was decreased by 28% ($P = 0.009$). The numbers of neutrophils and myeloperoxidase-positive cells per mm^2 thrombus area in the ticagrelor group were respectively decreased by 35% ($P = 0.016$) and 28% ($P = 0.047$), as compared with those in the clopidogrel group. Moreover, ticagrelor treatment reduced the ratio of monocytes number higher than 250 per mm^2 thrombus area compared with clopidogrel treatment (4% versus 29%, $P = 0.048$).

Conclusion In patients with undergoing PCI for STEMI, the loading dose ticagrelor regimen was associated with a reduction in inflammatory cell infiltration within thrombus compared with the loading dose clopidogrel regimen.

Keywords $P2Y_{12}$ receptor · Ticagrelor · Clopidogrel · Thrombus · Inflammation

Jing Shen, Guangzhong Liu, Guodong Wu and Xin Sun contributed equally to this work.

✉ Xin Sun
  sunxinflying@163.com

✉ Ye Tian
  yetian@ems.hrbmu.edu.cn

1 Shenzhen Cardiovascular Minimally Invasive Medical Engineering Technology Research and Development Center, Shenzhen People's Hospital(The Second Clinical Medical College, Jinan UniversityThe First Affiliated Hospital, Southern University of Science and Technology), Shenzhen, Guangdong 518020, China

2 Department of Cardiology, the First Affiliated Hospital, Cardiovascular Institute, Harbin Medical University, Harbin 150001, People's Republic of China

Introduction

Acute ST-segment elevation myocardial infarction (STEMI) is caused by the formation of thrombi in the coronary artery [1]. A coronary thrombus consists mainly of platelets, erythrocytes, and fibrin, and often contains inflammatory cells such as neutrophils and monocytes [2]. It has been demonstrated that inflammatory cells augment the growth of thrombus by interacting with platelets and erythrocytes [3]. Additionally, recent studies showed that thrombus inflammatory cell content was negative correlation with the perfusion of infarcted myocardium after primary percutaneous coronary intervention (PCI) in patients with STEMI [4, 5]. The importance of inflammatory cells in both the progression and prognosis of atherothrombotic disease raises the intriguing possibility of clinical benefit from drugs that inhibits the activation of inflammatory cells. The $P2Y_{12}$ receptor is

the target of antiplatelet drugs like the thienopyridine compounds ticlopidine, clopidogrel, and prasugrel or the direct, reversible antagonists ticagrelor and cangrelor [6]. Beyond the platelet physiology and pharmacology, previous studies have revealed the expression and roles of $P2Y_{12}$ receptor in inflammatory cells including microglial cells, [7] dendritic cells, [8] monocytes, [9] and unspecified leukocytes [10]. Moreover, the anti-inflammation benefits of $P2Y_{12}$ receptor inhibitors in atherosclerosis, [11] aneurysm, [12] ischemic brain injury, [13] and cardiac fibrosis [14] have also been observed. However, the impacts of $P2Y_{12}$ receptor inhibition in thrombus inflammation remain to be determined. Previous studies have revealed that ticagrelor provides more rapid, potent, and consistent $P2Y_{12}$ receptor inhibition than clopidogrel [15, 16]. The aim of this study was to evaluate the efficacy of $P2Y_{12}$ receptor inhibition with clopidogrel versus ticagrelor on inflammatory cell infiltration in thrombus aspirated from the STEMI patients.

Materials and methods

This clinical trial was a prospective, randomized, parallel design study undertaken between May, 2016, and February, 2017, in the First Affiliated Hospital of Harbin Medical University, Harbin City, China. All protocols were conformed to the Ethical Guidelines of the 1975 Declaration of Helsinki. The study was approved by the ethics committee of the First Affiliated Hospital of Harbin Medical University (201525) and registered at https://clinicaltrials.gov/ (NCT02639143).

Patients were eligible for inclusion in the trial if they had symptoms suggesting acute myocardial ischemia lasting more than 30 min, ST-segment elevation of more than 0.1 mV in two or more leads on the electrocardiogram (ECG), arrival at the hospital within 12 h of the onset of symptoms, and the intention to perform PCI. Exclusion criteria included an increased risk of bleeding or active bleeding, previous antiplatelet or anticoagulation therapy, and hemodynamic or electrical instability. All patients provided written informed consent.

Randomization was done with a 24-h computerized central automated voice response system. A computer-generated random allocation sequence was generated by the statistician for the Data Safety and Monitoring Board. Participants, those giving the interventions, those assessing outcomes, and those analyzing the data were masked to group assignment.

Patients who meet the inclusion criteria and none of the exclusion criteria will be consented. Initial background assessments include demographics, cardiovascular risk factors, relevant medical and surgical histories, clinical characteristics, and laboratory data. Immediately after randomization and before coronary angiography, patients randomly allocated clopidogrel received a 600-mg loading dose

followed by 75 mg once daily. Those allocated ticagrelor received a 180-mg loading dose followed by 90 mg twice daily. All patients received a 300-mg loading dose of aspirin followed by 100 mg daily during the treatment period. During PCI, thrombus aspiration was performed in the infarct-related artery with large thrombus burden (Thrombolysis In Myocardial Infarction thrombus score ≥ 3; or "cut-off" occlusion pattern and/or vessel diameter ≥ 3.5 mm) [17]. The infarct-related artery was defined as a major coronary artery perfusing an area compatible with the distribution of ST-segment elevation in the ECG.

Immunohistochemical analysis

Aspirated thrombi were immediately washed with saline, fixed in 10% formalin, and embedded in paraffin. The paraffin embedded samples were serially sectioned to produce 5-μm-thick sections. Every first and second section was stained with hematoxylin–eosin; the other sections were used for immunohistochemical staining of neutrophils (mouse monoclonal anti-CD11b, 1:500, Abcam, Hong Kong), monocytes (mouse monoclonal anti-CD14, 1:500, Abcam, Hong Kong), and myeloperoxidase (MPO) (mouse monoclonal anti-MPO, 1:500, Abcam, Hong Kong) [5]. The numbers of total inflammatory cells, neutrophils, MPO-positive cells, and monocytes per mm^2 thrombus area were calculated in eight random $600\times$ microscopic images. A pathologist performed the histopathological analyses while blinded to the groups.

High-sensitivity C-reactive protein analysis

Blood samples were drawn before and 24 h after PCI to measure serum high-sensitivity C-reactive protein (hs-CRP) level. The content of CRP in plasma was detected as per the manufacturer's instructions of the human high sensitivity CRP ELISA kit (Sigma, USA).

Power analysis

To estimate the group size, an observational pilot study of 22 patients treated with ticagrelor ($n=11$) or clopidogrel ($n=11$) was performed prior to the present study. This pilot study revealed that the patients treated with ticagrelor showed a significant improvement on the total inflammatory cells per mm^2 thrombus (836.60 ± 350.80 versus 1670.00 ± 720.00, $P<0.03$). With $\alpha=0.10$, two-tailed and a power of 85%, we needed 10 patients each group. Considering a compliance rate of 90%, we asked 22 patients to take part in our pilot study.

Statistical analysis

Continuous variables were described as mean $\pm$ standard deviation if it follows normal distribution, otherwise, as

median and interquantile; categorical variables were represented as count and percentage. A Shapiro–Wilk test was first used to test the normality of the data. Differences in continuous variables between groups were analyzed by t tests, and differences in categorical variables by χ^2 tests. A value of $P < 0.05$ was considered as statistically significant. Statistical evaluation was performed using Statistical Analysis System software (version 9.2, SAS institute, Cary, NC, USA).

Results

Patient characteristics

A total of 98 patients with STEMI were enrolled and randomly assigned treatment in this trial, of whom 55 underwent thrombus aspiration during PCI. Thrombus specimens were successfully aspirated from 49 patients. However, 2 specimens in ticagrelor group, despite serial sectioning, contained only minimal fragments of thrombus insufficient for histopathological analysis. Finally, 24 patients in the clopidogrel group and 23 in the ticagrelor group completed the study (Fig. 1). Baseline characteristics of the randomized patients are shown in Table 1. There were no differences in demographic characteristics of STEMI patients with between both groups. Time from the onset of chest pain to randomization was 205.42 ± 135.23 min in the clopidogrel group and 241.30 ± 139.98 min in the ticagrelor group. Time from randomization to thrombus aspiration was 78.79 ± 39.95 min in the clopidogrel group and 70.22 ± 28.60 min in the ticagrelor group (Table 1). Baseline characteristics were well matched between the two groups (Table 1).
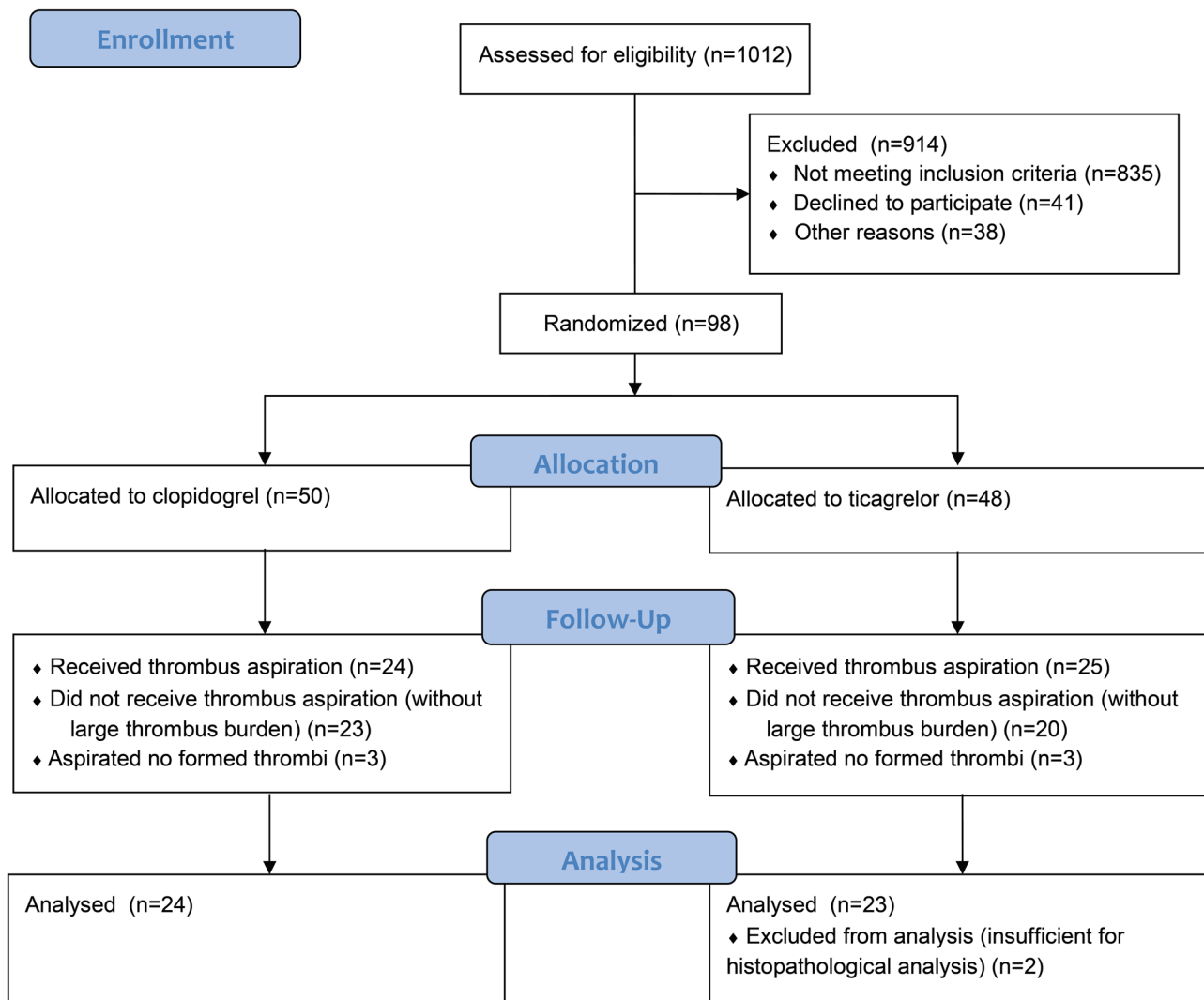

Fig. 1 Trial flow diagram

Table 1 Clinical characteristics

Characteristics	Clopidogrel ($n=24$)	Ticagrelor ($n=23$)	P
Age, y	52.54 ± 10.55	55.61 ± 12.17	0.36
Male, n (%)	22 (91.67%)	22 (95.65%)	1.00
Time from onset of chest pain to randomization, min	205.42 ± 135.23	241.30 ± 139.98	0.38
Time from randomization to thrombus aspiration, min	78.79 ± 23.32	70.22 ± 28.68	0.40
Statins before PCI, n (%)	9 (37.50%)	6 (26.00%)	0.53
Hypertension, n (%)	8 (33.33%)	12 (52.17%)	0.24
Diabetes, n (%)	9 (37.50%)	6 (26.00%)	0.53
Smoking, n (%)	18 (75.00%)	17 (73.91%)	1.00
OMI, n (%)	1 (4.17%)	0 (0%)	1.00
Stroke, n (%)	2 (8.33%)	4 (17.39%)	0.42
EF	51.63 ± 8.73	51.91 ± 8.31	0.91
Glucose, mmol/L	10.25 ± 4.78	9.54 ± 3.43	0.56
CK-MB, U/L	15.05 ± 21.96	33.96 ± 73.71	0.24
TnI, ng/mL	4.45 ± 13.23	2.86 ± 9.11	0.64
WBC in blood, $\times 10^9$/L	11.39 ± 3.04	12.72 ± 4.81	0.26
Neutrophils in blood, $\times 10^9$/L	9.34 ± 2.76	10.41 ± 4.54	0.33
Monocytes in blood, $\times 10^9$/L	0.45 ± 0.17	0.62 ± 0.37	0.05

Data are presented as mean ± standard deviation or number (%) and analyzed by t tests and χ^2 tests

PCI percutaneous coronary intervention, *OMI* old myocardial infarction, *EF* eject fraction, *CK-MB* creatine kinase-MB, *TnI* cardiac troponin I, *WBC* white blood cell

Detection of inflammatory cell infiltration of thrombus aspirated and hs-CRP

The primary endpoint of the number of total inflammatory cells per mm^2 thrombus area was 28% less in the ticagrelor group than in the clopidogrel group (1173.00 ± 561.00 versus 1639.00 ± 607.00, $P=0.009$, Fig. 2A and B). The numbers of neutrophils and MPO-positive cells per mm^2 thrombus area in the ticagrelor group were respectively decreased by 35% (209.30 ± 139.90 versus 321.20 ± 165.80, $P=0.016$, Fig. 3A and B) and 28% (238.90 ± 140.00 versus 332.60 ± 172.60, $P=0.047$, Fig. 3C and D), as compared

with those in the clopidogrel group. There was no difference in the number of monocytes per mm^2 thrombus area between the clopidogrel and ticagrelor groups (30.36 ± 41.84 versus 56.48 ± 80.83, $P=0.184$, Fig. 3E and F). However, ticagrelor treatment reduced the ratio of monocytes number higher than 250 per mm^2 thrombus area compared with clopidogrel treatment (4% versus 29%, $P=0.048$, Fig. 3G). The serum concentration of hs-CRP did not differ between the clopidogrel and ticagrelor groups neither before (6.23 ± 8.86 versus 6.49 ± 8.91, $P=0.94$) nor 24 h after PCI (18.11 ± 21.90 versus 13.69 ± 15.14, $P=0.49$) (Fig. 4).
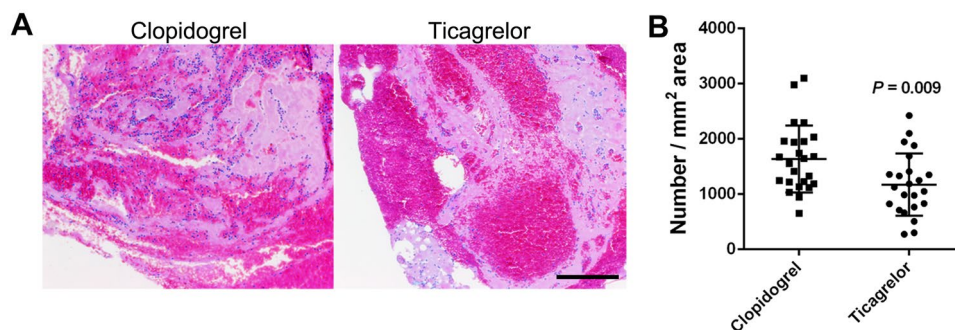

Fig. 2 Histological analysis of inflammatory cells within thrombi. **A** Representative histologic transverse sections stained with hematoxylin and eosin illustrate the presence of inflammatory cells within thrombi. Scar bar: 0.1 mm. **B** The numbers of inflammatory cells per mm^2 thrombus area were calculated (clopidogrel group: $n=24$; ticagrelor group: $n=23$). Data are presented as mean ± standard deviation and analyzed by t tests
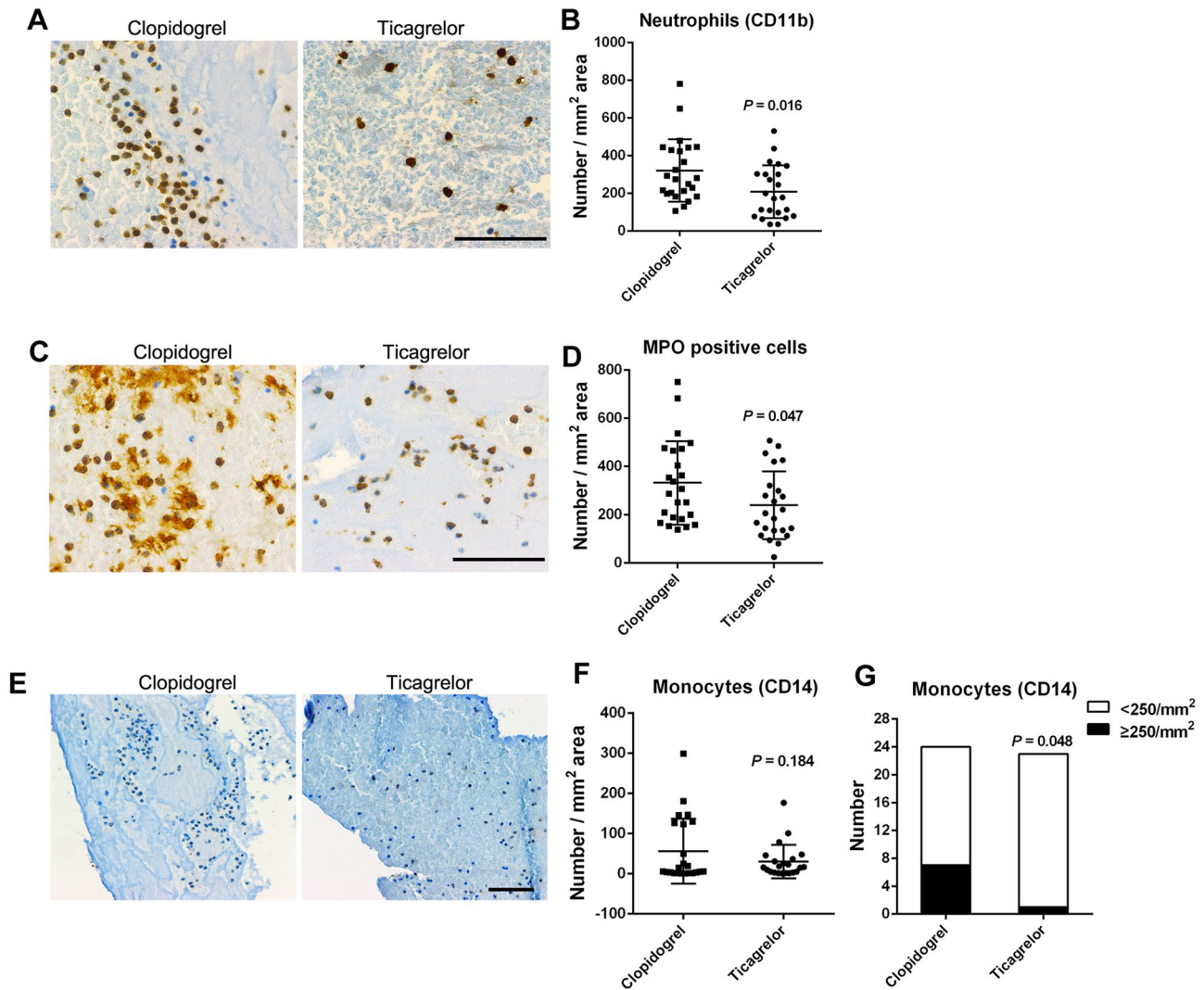
Fig. 3 Immunohistochemical analysis of neutrophils, MPO-positive cells, and monocytes within thrombi. **A** Representative immunohistochemical transverse sections stained with CD11b illustrate the presence of neutrophils within thrombi. Scar bar: 0.1 mm. **B** The numbers of neutrophils per mm^2 thrombus area were calculated (clopidogrel group: $n=24$; ticagrelor group: $n=23$). Data are presented as mean $\pm$ standard deviation and analyzed by t tests. **C** Representative immunohistochemical transverse sections stained with MPO illustrate the presence of MPO-positive cells within thrombi. Scar bar: 0.1 mm. **D** The numbers of MPO-positive cells per mm^2 thrombus area were calculated (clopidogrel group: $n=24$; ticagrelor group: $n=23$). Data are presented as mean $\pm$ standard deviation and analyzed by t tests. **E** Representative immunohistochemical transverse sections stained with CD14 illustrate the presence of monocytes within thrombi. Scar bar: 0.1 mm. **F** The numbers of CD14-positive cells per mm^2 thrombus area were calculated (clopidogrel group: $n=24$; ticagrelor group: $n=23$). Data are presented as mean $\pm$ standard deviation and analyzed by t tests. **G** The ratio of CD14-positive cells number higher than 250 per mm^2 thrombus area were calculated (clopidogrel group: $n=24$; ticagrelor group: $n=23$). Data were analyzed by χ^2 tests

Discussion

In this study, the major finding is that ticagrelor treatment provided greater attenuation of thrombus inflammatory cell infiltration than clopidogrel treatment in STEMI patients. The P2 purinergic receptors consist of two families: the ionotropic receptors (P2X) contain channels that permit ion flow, whereas the metabotropic receptors (P2Y) are G-protein coupled second messenger systems [18]. In inflammatory cells, P2Y receptors are involved in pro-inflammatory responses,

[19] migration [20], and phagocytosis [21]. A substantial body of literature [9, 22] has demonstrated that the $P2Y_{12}$ receptor plays a pivotal role in inflammatory cell migration when activated by its ligands, ATP and ADP. Webster et al. reported that $P2Y_{12}$ deficiency or inhibition by clopidogrel attenuated microglia migration in response to injured neurons [22]. Kronlage et al. demonstrated that monocytes from mice deficient in $P2Y_{12}$ exhibited impaired chemotaxis [9]. In agreement with these reports, we showed in this study that pharmacological $P2Y_{12}$ receptor inhibition by clopidogrel
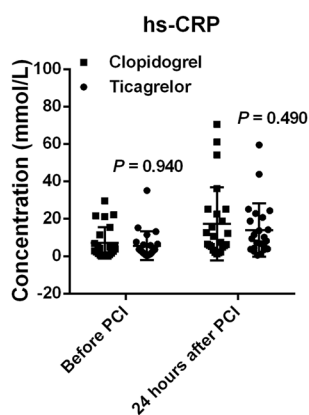
Fig. 4 The concentration of hs-CRP. The serum concentration of hs-CRP was detected before and 24 h after PCI (clopidogrel group: $n = 24$; ticagrelor group: $n = 23$). Data are presented as mean ± standard deviation and analyzed by t tests

and ticagrelor was protective against inflammatory cell infiltration in thrombus.

However, it is still possible that the effect of clopidogrel and ticagrelor seen on thrombus inflammation could be due to its direct effect on platelets. Platelets are an important source of pro-inflammatory mediators and cytokines that can recruit inflammatory cells such as neutrophils and monocytes [23]. In addition, activated platelets have been shown to stimulate platelet-leukocyte interactions, which are involved in the pathogenesis of several inflammatory diseases. Xiao et al. showed that in patients with NSTEMI, platelet-monocyte and platelet-neutrophil conjugates in whole blood were significantly reduced by clopidogrel treatment [24]. In a murine model of abdominal aortic aneurysms, Liu et al. reported that inhibition of platelet substantially decreased monocytes recruitment into aneurysm tissue [12]. Moreover, activation of $P2Y_{12}$ receptor has been shown to be involved in the formation of platelet-leukocyte aggregates at the site of the growing thrombus [25]. These studies suggest that platelet $P2Y_{12}$ receptor inhibition likely contributes to clopidogrel and ticagrelor-induced attenuation of inflammatory cell infiltration in thrombus by interrupting platelet-monocyte and platelet-neutrophil conjugates.

Infiltration of inflammatory cells contributes to the evolution of thrombus formation and growth. It has been reported that activated neutrophils and monocytes induce alterations of erythrocyte properties, which result in hyperaggregability, and increase the expression of tissue factor activity, leading to a thrombotic state [26, 27]. Furthermore, inflammatory cell infiltration could lead to poor procedural outcome during PCI in patients with STEMI. Arakawa et al. demonstrated that high neutrophil density in an aspirated thrombus is associated with impaired coronary microcirculation and left ventricular dysfunction [28]. Additionally, Yunoki

et al. showed that the thrombi aspirated in patients with distal embolization contained more myeloperoxidase-positive cells (neutrophils and monocytes) than thrombi aspirated in patients without distal embolization [4, 5].

Ticagrelor is a direct-acting inhibitor of $P2Y_{12}$ receptor, providing more rapid, potent, and consistent $P2Y_{12}$ receptor inhibition than clopidogrel [15]. This support our results that the thrombus inflammatory cell infiltration in patients receiving ticagrelor treatment was less than that in patients receiving clopidogrel treatment. Such anti-inflammation effects may, at least in part, explain the previous findings in the PLATO trial that ticagrelor treatment provided greater mortality benefit than clopidogrel treatment in STEMI patients [29].

Routine thrombus aspiration is not recommended by current guidelines, but in cases of large thrombus burden after opening the vessel with a guide wire or a balloon, thrombus aspiration may be considered [30]. In this case, thrombus aspiration was performed in 55 of 98 patients with large thrombus burden in the infarct-related artery. At the time of patient enrollment (2016–2017), international guidelines recommended prasugrel or ticagrelor rather than clopidogrel as first-line antiplatelet strategy for STEMI patients. However, guidelines of China recommended either clopidogrel or ticagrelor as first-line antiplatelet strategy for STEMI patients. In accordance with this, patients with STEMI were randomly assigned to receive clopidogrel or ticagrelor.

Study limitations

Despite the encouraging findings, our study has several limitations. First, it involved a relatively small number of patients and was conducted at a single center. Second, the small sample size of thrombus was insufficient for further western blotting and real-time PCR analysis. Third, the level of inflammatory factors in both patients was not determined. The dropout rate in both groups after randomization was high. Therefore, further larger-scale studies are needed to validate the clinical effect of ticagrelor in patients with STMEI. Finally, our study was not powered to detect clinical long-term safety differences between the 2 treatment groups.

Conclusions

In conclusion, our findings in this study indicate that the loading dose ticagrelor regimen was associated with a reduction in inflammatory cell infiltration within thrombus compared with the loading dose clopidogrel regimen in patients with undergoing PCI for STEMI.

Author contribution Jing Shen, Guang Zhong Liu, Guo Dong Wu, Xin Sun, and Ye Tian designed and conducted the experiment; Jing Shen, Guang Zhang Liu, and Guo Dong Wu.analyzed the data and wrote the manuscript. Lijian Sheng, Zhengyu Cao, Shu Yuan Guo, and Shao Hong Dong conducted the experiments. Jing Shen, Guang Zhong Liu, and Xin Sun revised the manuscript; and all authors approved the final version of the manuscript.

Declarations

Ethical approval The study was approved in advance by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University and was conducted in accordance with International Conference on Harmonization Guideline for Good Clinical Practice and Declaration of Helsinki.

Informed consent All subjects provided written informed consents before their participation.

Conflict of interest The authors declare no competing interests.

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