Ticagrelor does not affect left ventricular remodeling following acute myocardial ischemia

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INTRODUCTION

Platelets play an important role in early thrombotic events and late neointimal formation after arterial injury such as percutaneous coronary intervention (PCI) and plaque rupture in acute coronary syndrome (ACS) [1,2]. Inhibition of the adenosine diphosphate (ADP)-activated P2Y12 receptor in platelets by clopidogrel in combination with aspirin has been shown to reduce ischemic events after ACS and PCI [3]. Clopidogrel is a thienopyridine derivative whose active metabolites are known inhibitors of ADP-induced platelet aggregation. It is a prodrug that requires activation by specific hepatic cytochrome P450 isoenzymes to become an active metabolite. This two-step activation process results in a delayed onset of action and is susceptible to the influence of genetic polymorphisms in P450 isoenzymes, resulting in differential therapeutic responses and varied thrombotic events [4]. Recently, third-generation P2Y12-receptor inhibi-
itors have been shown to overcome many of the pharmaco-
dynamic limitations associated with clopidogrel treatment
and have resulted in improved outcomes in patients with
ACS. Ticagrelor, the first nonthienopyridine direct P2Y12-
receptor inhibitor, provides better clinical outcomes than
clopidogrel in patients with ACS [5]. Ticagrelor reduced both
vascular disease and any cause of death, as well as throm-
botic complications regardless of revascularization. These
effects could not be explained by faster and more potent
platelet inhibition alone [6]. In animal studies, ticagrelor re-
duced postinfarction myocardial damage through reduced
expression of inflammatory markers, attenuation of infarct
size and fibrosis, and favorable effects on left ventricular (LV)
remodeling [7-9]. However, ticagrelor proved to be no more
effective in patients with ACS when compared with other
P2Y12-receptor inhibitors [10,11]. These conflicting results
raise the question of whether the benefit of these more po-
tent agents would be felt at a level of platelet inhibition sim-
ilar to that seen with thienopyridine drugs. To address this
problem, we used a porcine model to compare the ther-
apeutic efficacy of ticagrelor and clopidogrel at equivalent
levels of platelet inhibition after acute myocardial infarction
(AMI).

MATERIALS AND METHODS

Animal preparation

We used 20 Yorkshire × Landrace F1 crossbred castrated
boars weighing 30-35 kg each. All animals were housed
under controlled environmental conditions and under pro-
fessional veterinary care. Individual animals were grouped
according to the administered P2Y12-receptor inhibitors:
i.e. either ticagrelor or clopidogrel. All study subjects were
on loading doses of dual antiplatelet agents (300 mg aspi-
rin plus 600 mg clopidogrel or 300 mg aspirin plus 180 mg
ticagrelor). These drugs and dosages correspond to those
used in the Platelet Inhibition and Patient Outcomes (PLA-
TO) trial and were administered 2 hours for ticagrelor and 4
hours for clopidogrel before the procedure [5]. The dosages
of ticagrelor and clopidogrel were selected because they
have been shown to be achieved similar levels of inhibition
of ADP-induced platelet aggregation [9]. Following experi-
ment, the animals received the corresponding maintenance
doses of the antiplatelet agents (90 mg ticagrelor twice
daily or 150 mg clopidogrel per day). Platelet function was
monitored using the VerifyNow P2Y12 assay (Accumetrics
Inc., San Diego, CA) at baseline and 2 weeks. For the P2Y12
assay, percent inhibition of P2Y12 reaction units (PRU) was
calculated for each subject as follows. Percent inhibition =
(Baseline PRU - Post drug PRU) / Baseline PRU) × 100. The
protocol is described in Fig. 1.

Anesthesia

All pigs were fasted for 24 h before the procedure with
unlimited access to water. On the day of the procedure, pigs
were anesthetized with zolazepam and tiletamine (2.5 mg/
kg each, Zoletil50®, Virvac, Caros, France), xylazine (3 mg/
kg, Rompun®, Bayer AG, Leverkusen, Germany), and azap-
erone (6 mg/kg, Stresnil®, Janssen-Cilag, Neuss, Germany).
Intravenous fluid administration with sterile 0.9% saline via
a marginal ear vein was given continuously throughout the
experiment. After intubation, anesthesia was maintained
with inhalation anesthesia using sevoflurane (1%) in oxygen
(100%). All pigs were ventilated mechanically.

Loop recorder implantation

After anesthesia, a loop recorder (Reveal LINQ®; Medtron-
ic, Inc., Minneapolis, MN) was implanted for detecting ven-
tricular arrhythmia. An implantable loop recorder (ILR) was
implanted subcutaneously into the left pectoral region [12].
A small incision was made and the subcutaneous tissues
were dissected to the muscle level. The cleavage plane be-
tween the left pectoralis muscles was gently dissected man-
ually. Once the ILR had been inserted into the submuscular

Fig. 1. Scheme of the study protocol. AMI: acute myocardial infarction. 1: P2Y12 inhibitor loading – G1 ticagrelor, G2 clopidogrel, 2: baseline 2D
echocardiography, implantation of loop recorder, EGM recording, blood sampling, 3: coronary angiogram, induction of acute myocardial infarction (AMI), EGM
recording, 4: monitor for spontaneous arrhythmias for 1 hours, EGM recording, blood sampling, 5: maintenance of antiplatelet agent (aspirin, clopidogrel or
ticagrelor) for 2 weeks, 6: follow-up coronary angiogram, EGM recording, blood sampling, measurement of myocardial infarct size, analysis of loop recorder.
pocket, the amplitude of spontaneous electrocardiogram R waves was measured through the device’s telemetry. The device was then anchored through the two suture holes of the header and the surgical wound was sutured. The electrodes were oriented outward. The basic requirements for subpectoral implantation were an R-wave amplitude of at least 0.3 mV and a peak-to-peak R-wave amplitude at least twice the peak T- and P-wave amplitudes. R-wave amplitudes were determined at implantation and during follow up. These were defined as the mean value of a manual peak-to-peak measurement of at least five consecutive signals. No complications occurred during implantations. Data were stored automatically when arrhythmic events corresponded to the following preprogrammed criteria: sinus arrest as a pause >3 s, ventricular tachycardia (VT) as a heart rate >300 bpm and lasting <30 s, and sustained VT as lasting >30 s [13].

**Two-dimensional (2-D) echocardiography**

All animals underwent 2-D transthoracic echocardiography at baseline (before the procedure), and 2 weeks after the induction of AMI. Cardiac images were taken using an echocardiography system (Vivid S5, GE Healthcare, Schenectady, NY, USA) under general anesthesia while the pigs were in the supine position. The LV ejection fraction, LV end-systolic volume, and LV end-diastolic volume were determined using a modified biplane Simpson’s rule in 2- and 4-chamber views.

**Induction of myocardial ischemia**

A 7-Fr arterial sheath was placed in the left carotid artery under local anesthesia with 2% lidocaine. After infusion of 10,000 U of heparin, a 7-Fr coronary artery guiding catheter was placed within the opening of the artery and a baseline coronary angiogram was obtained under fluoroscopic guidance using a mobile C-arm (BV-25 Gold; Philips BV, Eindhoven, The Netherlands). Myocardial ischemia was induced by embolization using occluder to interrupt intravascular blood flow in the middle left anterior descending artery (LAD), just distal to the second diagonal branch or the septal branch (Fig. 2) [14]. The hole of occluder which wire passes, kept blood flow. Continuous electrocardiographic monitoring was performed to confirm normal ST segments at baseline and ST elevation during the ischemic period and to monitor any occurrence of cardiac arrhythmia. All ventricular arrhythmias were terminated by DC cardioversion and defibrillation (Biphasic 200J). Each animal was carried back to the holding facility and monitored until recovery.

**Follow-up angiogram, echocardiography, and pathology**

Two weeks later, animals underwent follow-up 2-D transthoracic echocardiography and coronary angiography in the same orthogonal views to check for vessel patency. At the end of the experiment, pigs were anesthetized and euthanized with an overdose of potassium chloride. The extracted heart was rinsed and myocardial sections, including the left and right ventricle were obtained at 1-cm intervals using

![Fig. 2. Preparation of PET occlusion. (A) A representative image of the actual experiment. The balloon catheter was inserted at the rear of the PET occlusion. (B) Baseline coronary angiogram. (C) Coronary angiogram showing PET embolism in the middle LAD including the remaining TIMI III flow. Yellow arrow indicates the PET embolism. PET: polyethylene terephthalate, LAD: left anterior descending artery.](image-url)
a microtome knife. The myocardial sections were incubated in 2,3,5-triphenyl tetrazolium chloride (TTC) solution until portions of viable myocardium turned brick red. The specimens were reviewed by two cardiac pathologists for pathological changes in the infarcted myocardium. Infarct size was estimated on digital photographs of TTC-stained sections by outlining the LV area and the TTC negative infarct area. Infarct size was then expressed as the percentage of the LV area.

Statistical analysis
Baseline characteristics are summarized as the mean ± standard deviation (SD) for continuous variables, and as frequencies with percentages for categorical variables. Comparisons between the two groups were analyzed with Student’s t or Mann-Whitney nonparametric U tests for continuous variables, and chi-square or Fisher’s exact tests for categorical variables as appropriate. Event rate curves were obtained by Kaplan-Meier analysis and compared using the log-rank test. All statistical tests were two-sided and performed using IBM SPSS Statistics software (version 24.0; IBM Corp., Armonk, NY, USA). p values < 0.05 were considered significant.

Ethics statement
This animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-H-2013-18), and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

RESULTS
A total of 20 pigs were anesthetized and prepared for this study. Nine pigs died within 24 hours. No additional deaths occurred during the 2 weeks. Therefore, a final total of 11 pigs were analyzed.

Platelet function assay
The response of platelet aggregation inhibition to ticagrelor and clopidogrel showed a variable distribution with mean and standard deviation. At baseline and 2 weeks later, VerifyNow P2Y12 data expressed in P2Y12 reaction units (PRU inhibition percent) are presented in Fig. 3. No significant differences in platelet aggregation inhibition were observed between the two groups (at baseline and 2 weeks later).

ILR data analysis
An ILR was implanted subcutaneously into the left pectoral region successfully in all pigs. The mean R-wave amplitude was 1.21±0.31 mV. No complications occurred during implantation of the ILR. Ventricular fibrillation (VF) occurred within 24 hours after the infarction in nine pigs according to analysis of the ILR data. All nine of these pigs died. The incidence of arrhythmic death due to VF was not different between the two groups (40% vs. 50%; p=0.886) (Fig. 4).

Two-dimensional echocardiography results
The 2-D echocardiographic study revealed no difference in LV function or volumes at baseline (Table 1). At 2 weeks, the LV ejection fraction (%) was significantly lower than at baseline in both groups, but there was no significant difference between the groups (44.6±7.4% in the ticagrelor group vs. 36.9±5.7% in the clopidogrel group; p=0.091).

Histopathology
TTC staining clearly showed a well-defined white area of infarction involving the cardiac apex and LV septal wall. The white infarcted myocardium contrasted with the red color of...
the surrounding normal cardiac muscle. The excised hearts in the 11 surviving pigs showed that occlusion of LAD resulted in large ischemic areas of similar size (Fig. 5). There was no significant difference in the myocardial mass of the ischemic area (%LV area) between the two groups (13.8±3.3% in the ticagrelor group vs. 16.2±4.4% in clopidogrel group; p=0.286).

**DISCUSSION**

To the best of our knowledge, this is the first study to evaluate the relationship between type of P2Y12-receptor inhibitors and cardiac function in a porcine chronic ischemic heart model. Disappointingly, at a similar level of platelet inhibition, the combination of aspirin with ticagrelor did not reduce mortality or improve cardiac function compared with the combination of aspirin with clopidogrel.

In this study, we measured platelet response to ticagrelor and clopidogrel by using the VerifyNow P2Y12 assay. The VerifyNow P2Y12 is a reliable assay that tests platelet activity and uses a combination of ADP and prostaglandin E1 to directly measure the effects of clopidogrel on the P2Y12 receptor [15]. Validation of this assay has been performed in various clinical settings and has been approved by the US Food and Drug Administration. In accordance with the dosage used in the PLATO trials, loading doses of dual antiplatelet agents (300 mg aspirin plus 600 mg clopidogrel or 300 mg aspirin plus ticagrelor 180 mg) were given 4 hours before the procedure in the present study. These ticagrelor

![Graph of survival probability](image-url)

**Table 1.** Two-dimensional echocardiography results

|                  | Ticagrelor | Clopidogrel | p value |
|------------------|------------|-------------|---------|
| Baseline n=10    | LVEF (%)   | 58.3±1.9    | 60.2±4.4| 0.405   |
|                  | LVEDV index (mL/m²) | 54.9±14.5 | 56.2±7.8| 0.801   |
|                  | LVESV index (mL/m²) | 21.2±5.5  | 24.3±2.5| 0.131   |
| 2 weeks n=6      | LVEF (%)   | 44.6±7.4    | 36.9±5.7| 0.091   |
|                  | LVEDV index (mL/m²) | 62.6±6.9  | 71.8±23.5| 0.378 |
|                  | LVESV index (mL/m²) | 40.7±9.6  | 41.9±4.78| 0.801 |

AMI: acute myocardial infarction, LVEDV: left ventricular end-diastolic volume, LVEF: left ventricular ejection fraction, LVESV: left ventricular endsystolic volume.
and clopidogrel doses were selected because they achieved similar inhibition of ADP-induced platelet aggregation. We confirmed that the loading dose and the maintenance doses achieved a significant and sustained antiplatelet effect in both groups at the time of experiment and at 2 weeks (Fig. 3).

At a similar level of platelet inhibition, we found no significant difference in ventricular arrhythmia and cardiac dysfunction between the two groups in our study. About half of the pigs died because VF occurred within 24 hours. Ventricular arrhythmias are common after acute myocardial infarction and are a marker of electrical instability that identifies subjects at increased risk of arrhythmogenic death. Some studies have suggested that ticagrelor could induce earlier and more complete inhibition of platelets leading to a lower thrombus burden and improve ventricular arrhythmia and cardiac function compared with clopidogrel [16]. Ticagrelor reduced vascular and any cause of death as well as thrombotic complications regardless of revascularization [6]. In animal studies, ticagrelor has been shown to be more cardioprotective than clopidogrel. This finding has been attributed to ticagrelor’s unique ability to raise tissue adenosine by blocking the equilibrative nucleoside transporter I. Ticagrelor reduced postinfarction myocardial damage compared with clopidogrel through reduced expression of inflammatory markers, attenuation of infarct size and fibrosis, and favorable effects on LV remodeling [7-9]. At a similar level of platelet inhibition, however, we found that ventricular arrhythmia and LV remodeling were not different between the two groups over a 2-week period. The reasons for this are unclear since several studies have reported the existence of dose- and time-dependent interindividual differences in the antiplatelet response to clopidogrel. Clopidogrel is a prodrug and must be enzymatically activated by a slow, multistep process involving hepatic cytochrome p450 enzymes. When it is given just prior to reperfusion in animal models, it is not protective. However, if sufficient time is allowed for conversion to its active form as evidenced by blockade of platelet reactivity, then clopidogrel does become cardioprotective.

Second, in our study, aspirin loading may have had an effect. In the clinical practice, dual antiplatelet therapy consisting of aspirin and P2Y12-receptor inhibitors, has remained the cornerstone of treatment for patients with AMI. Inhibition of the adenosine diphosphate (ADP) P2Y12 receptor by clopidogrel, in combination with aspirin, has been shown to reduce ischemic events after ACS and PCI compared with a P2Y12-receptor inhibitor alone. However, ticagrelor-related cyclooxygenase 2/prostaglandin I2 cardioprotective effects might be acutely abolished on aspirin loading [17]. Moreover, ticagrelor shows reduced efficacy in ACS patients where higher maintenance doses of aspirin (>300 mg) are more widely prescribed [18].

Third, ticagrelor has been shown to offer protection against myocardial infarction in animal models of transient coronary occlusion (reperfusion model). But in the chronic ischemic heart failure model used in our study, ticagrelor did not affect the chronic occlusion models. Small sample size could also introduce bias to the results of the statistical analysis. Almost half of the pigs in our study died of ventricular arrhythmia, which may not have sufficient power
to detect therapeutic differences in the actions of ticagrelor and clopidogrel.

In the present study, we did not find any difference in ventricular arrhythmia and cardiac dysfunction between animals treated with ticagrelor and clopidogrel in a chronic ischemic heart failure model. Regardless of whether ticagrelor has pleiotropic effects, we agree that ticagrelor is a superior drug compared to clopidogrel in the setting of reperfusion therapy for AMI. The latter suffers from an onset of effect that is too slow for acute treatment as well as a common genetic variation that negates its metabolic activation in a significant portion of the patient population. Further randomized trials are needed to evaluate the pleiotropic effects of ticagrelor in the long term.

In conclusion, ticagrelor did not significantly reduce ventricular arrhythmia or cardiac dysfunction compared with clopidogrel at a similar level of platelet inhibition in a chronic ischemic heart failure model.

ACKNOWLEDGEMENTS

The authors thank the collaborating laboratory personnel for their excellent technical assistance in this study. This study was supported by research funds from the Basic Science Research Program through the National Research Foundation of Korea (NRF-2018R1D1A1B07040783).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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