Translation of experimental cardioprotective capability of $P2Y_{12}$ inhibitors into clinical outcome in patients with ST-elevation myocardial infarction

Short title: Clinical impact of $P2Y_{12}$ inhibitors´ cardioprotective capability

Marie V. Hjortbak[1]; Kevin K.W. Olesen[2]; Jacob M. Seefeldt[1], Thomas R. Lassen[1], Rebekka V. Jensen[2], Alexander Perkins[3], Matthew Dodd[3], Tim Clayton[3], Derek Yellon[4], Derek J. Hausenloy[4,5,6,7], Hans Erik Bøtker[1,2] on behalf of the CONDI-2/ERIC-PPCI investigators

Affiliations:

1. Department of Clinical Medicine, Cardiology, Aarhus University, Aarhus, Denmark
2. Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
3. London School of Hygiene and Tropical Medicine, Clinical Trials Unit, London, UK
4. The Hatter Cardiovascular Institute, University College London, London, UK
5. Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore
6. National Heart Research Institute Singapore, National Hearts Centre, Singapore Yong Loo Lin School of Medicine, National University Singapore, Singapore
7. Cardiovascular Research Center, College of Medical and Health Sciences, Asia University, Taiwan

Address for correspondence:

Marie V. Hjortbak

Aarhus University, Department of Clinical Medicine

Palle Juul-Jensens Boulevard 82, DK-8200, Aarhus N, Denmark

Phone: 0045 78452262

Email: hjortbak@clin.au.dk

Abstract

Objectives: We studied the translational cardioprotective potential of $P2Y_{12}$ inhibitors against acute myocardial ischemia/reperfusion injury (IRI) in an animal model of acute myocardial infarction and in patients with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI).

Background: $P2Y_{12}$ inhibitors have pleiotropic effects that may induce cardioprotection against acute myocardial IRI beyond their inhibitory effects on platelet aggregation.

Methods: We compared the cardioprotective effects of clopidogrel, prasugrel and ticagrelor on infarct size in an *in vivo* rat model of acute myocardial IRI, and investigated the effects of the $P2Y_{12}$ inhibitors on enzymatic infarct size (48-hour area-under-the-curve (AUC) troponin T release) and clinical outcomes in a retrospective study of STEMI patients from the CONDI-2/ERIC-PPCI trial using propensity score analyses.

Results: Loading with ticagrelor in rats reduced infarct size after acute myocardial IRI compared to controls (37±11% vs 52±8%, $p<0.01$), whereas clopidogrel and prasugrel did not (50±11%, $p>0.99$ and 49±9%, $p>0.99$, respectively). Correspondingly, troponin release was reduced in STEMI patients treated with ticagrelor compared to clopidogrel (adjusted 48-hour AUC ratio: 0.67, 95% CI 0.47-0.94). Compared to clopidogrel the composite endpoint of cardiac death or hospitalization for heart failure within 12 months was reduced in STEMI patients loaded with ticagrelor (HR 0.63; 95% CI 0.42-0.94) but not prasugrel (HR 0.84, 95% CI 0.43-1.63), prior to PPCI. Major adverse cardiovascular events did not differ between clopidogrel, ticagrelor or prasugrel.

Conclusions: The cardioprotective effects of ticagrelor in reducing infarct size may contribute to the clinical benefit observed in STEMI patients undergoing PPCI.

Key words: $P2Y_{12}$ inhibitor, cardioprotection, ischemic conditioning, myocardial infarction

Introduction

Acute myocardial infarction still contributes to mortality and morbidity worldwide. During myocardial infarction, the myocardium suffers ischemic damage, which can only be targeted by timely reperfusion therapy. The paradoxical myocardial reperfusion injury that may extend final infarct size [77] requires adjunctive treatment strategies beyond reperfusion to improve clinical outcome. Although remote ischemic conditioning (RIC) reduces myocardial injury by activating inherent cardioprotective mechanisms [34], verification of a clinical benefit for the patients has been challenging, mainly because clinical event rates with modern reperfusion therapy are low [27, 29, 43].

The cardiomyocyte has been the primary target of cardioprotective strategies given that final infarct size is the main predictor of cardiovascular mortality. However, increasing evidence shows that other targets might be of importance to attenuate injury during myocardial infarction. In addition to mediating the occlusive thrombus in acute myocardial infarction, platelets may also release factors that exacerbate acute myocardial ischemia and reperfusion injury [22, 79].

Loading treatment with $P2Y_{12}$ inhibitors is an established adjunctive therapy to invasive treatment of acute coronary syndrome because of their inhibitory effect on platelet aggregation. However, clopidogrel, prasugrel and ticagrelor have all demonstrated pleiotropic, cardioprotective effects in experimental studies [72, 74]. Observations from minor, retrospective studies indicate that the cardioprotective effects of $P2Y_{12}$ inhibitors may be transferrable to a clinical setting [36, 52].

The aims of the present study were to compare head-to-head loading with clopidogrel, prasugrel and ticagrelor on infarct size in an experimental rat model of myocardial ischemia and reperfusion, and subsequently study the translational potential in a cohort of STEMI patients from the CONDI-2/ERIC-PPCI trial [29].

Methods

Rat experiments

All animal experiments were performed in accordance with Danish legal and institutional guidelines (Authorization number: 2018-15-0201-01475). Male Sprague Dawley rats (Taconic, Ry, Denmark) (250-350 g) were randomized to one of the following protocols: 1) Control, 2) IPC, 3) RIC, 4) Clopidogrel, 5) Prasugrel, 6) Ticagrelor, 7) IPC+Ticagrelor or 8) RIC+Ticagrelor as specified in Figure S1. Combination therapy with ischemic conditioning and ticagrelor was investigated to determine interactions.

Delivery of $P2Y_{12}$ inhibitors

$P2Y_{12}$ inhibitors were administered by oral gavage using crushed tablets suspended in tab water; doses were adjusted to body weight of the individual rat. Clopidogrel (15 mg/kg) (Clopidogrel STADA, STADA Arnzneimittel AG, Bad Vilbel, Germany) was given 4 hours prior to induction of myocardial ischemia, ticagrelor (20 mg/kg)(Brilique, AstraZeneca, Cambridge, United Kingdom) and prasugrel (10 mg/kg) (Efient, Daiichi-Sankyo Europe GmbH, Munich, Germany) were given 2 hours prior to induction of myocardial ischemia. Placebo treatment consisted of tab water only given 2 hours before myocardial ischemia.

The dosage and timing of $P2Y_{12}$ inhibitors were chosen from available data in the literature. Clopidogrel is a prodrug that requires enzymatic activation. The loading dose of clopidogrel must be given before reperfusion of the myocardium, but the duration of pretreatment to induce protection varies between 4 hours and two days in animal studies [66, 74]. In the present study we loaded the animals with clopidogrel 4 hours prior to induction of ischemia because the resultant plasma concentration is associated with antiplatelet efficacy [56, 66] and because the approach may have some potential for clinical translation when given before reperfusion. The dose of clopidogrel

was based on previous studies demonstrating cardioprotective effect of clopidogrel [66, 76]. Ticagrelor and prasugrel have more rapid and potent antiplatelet responses than clopidogrel. In rats, platelet aggregation is significantly inhibited one to two hours after administration of ticagrelor or prasugrel, whereas clopidogrel may require 2-4 hours [56, 57]. This pharmacologic profile may increase the cardioprotective potential of ticagrelor and prasugrel within a clinically relevant timeframe for STEMI patients. As for clopidogrel, the doses of prasugrel [25, 58] and ticagrelor [3, 66, 72, 76] were based on previous studies demonstrating cardioprotective effect.

In vivo myocardial infarction

The rats were anesthetized with an intraperitoneal injection of pentobarbiturate (100 mg/kg body weight) (Skanderborg Pharmacy, Skanderborg, Denmark). Immediately after anesthesia was achieved the rats were intubated, connected to a ventilator (UGO BASILE, Comerio, Varese, Italy), and ventilated with atmospheric air. Body temperature was maintained at 37 °C (±0.5 °C) (CMA/150, CMA Microdialyses AB, Krista, Sweden). The heart was accessed through a left sided thoracotomy. The left anterior descending artery (LAD) was identified and ligated with a 4-0 silk suture (Sofsilk™, Covidien, Dublin, Ireland) at the level of the left atrial appendix tip. All hearts received 30 minutes of myocardial ischemia followed by 2 hours of reperfusion.

RIC was performed prior to the thoracotomy using a tourniquet around a hind leg, to induce 3 cycles of 5 minutes limb ischemia followed by 5 minutes of reperfusion. IPC was performed after the thoracotomy, using the myocardial suture around LAD to induce 3 cycles of 5 minutes of ischemia followed by 5 minutes of reperfusion.

Infarct size

After 2 hours of reperfusion, the LAD was reoccluded, and a 2 % solution of Evans Blue (Sigma-Aldrich, St. Louis, MO, USA) was injected in the inferior vena cava to visualize the area at risk. The hearts were rapidly removed and stored at -80 °C. The hearts were then sliced and stained using a 1% solution of Triphenyl Tetrazolium Chloride (Sigma-Aldrich, St Louis, MO, USA). After 24 hours in 4% formalin buffer (VWR International, Leuven, Belgium), the slices were scanned using a flatbed scanner (Epson Perfection V600 Photo scanner, Epson, Nagano, Japan). The infarct size, area at risk and area of the left ventricle were assessed using ImageJ software (NIH, Bethesda, Maryland, USA). All measurements were correlated to the wet weight of the individual slice. Final infarct size is expressed as the percent of infarcted area over the area at risk.

Statistical analyses

Statistical analyses of the rat experiments were performed using GraphPad Prism 8.2.0 (GraphPad Software, California, USA). Data are presented as mean ± SD. One-way ANOVA with post hoc Bonferroni correction for multiple comparisons was used for all rat experimental data [13]. Sample size calculations were based on an infarct size of 50% in controls and 35% in intervention groups, with a standard deviation of 10%. A significance level α=0.05 and a power of 95% yielded a sample size of 12 animals in each group.

We tested for interaction between type of intervention (none, IPC, and RIC) and ticagrelor on infarct size. The interaction analysis was performed in StataIC version 16 (Stata Corp, College Station, Texas, USA).

Clinical studies

The clinical part of the study was designed as a retrospective, non-prespecified post hoc sub-study of the international, multicenter, single-blind, randomized controlled CONDI-2/ERIC-PPCI trial [29]. A detailed description of the study is provided in the original publication [29]. Patients with ST-segment elevation myocardial infarction, eligible to PPCI, were randomized to standard treatment or treatment with RIC. The study included patients from 33 centers across United Kingdom, Denmark, Spain and Serbia. We analyzed the data collected for the CONDI-2/ERIC-PPC trial to investigate interaction between treatment with $P2Y_{12}$ receptor inhibitors and RIC in relation to PPCI for clinical outcomes.

In accordance with contemporary guidelines, patients with STEMI were loaded with a $P2Y_{12}$ receptor inhibitor prior to PPCI. Patients received either clopidogrel (600mg), ticagrelor (180 mg) or prasugrel (60 mg). Choice of $P2Y_{12}$ receptor inhibitor for loading was based on current guidelines and regional preferences. The time from administration of the chosen $P2Y_{12}$ inhibitor to reperfusion by PPCI was not registered.

Patient Selection

We excluded patients, who were on treatment with clopidogrel, ticagrelor, or prasugrel prior to PPCI. Patients, who were not treated with either peri-procedural clopidogrel, ticagrelor or prasugrel, were also excluded.

Infarct size

We estimated myocardial infarct size measured as area-under-the-curve (AUC) of high-sensitivity troponin T measured between 0 and 48 hours after PPCI in a subset of patients.

Clinical outcomes

The main endpoint was a composite of cardiac death or hospitalization for heart failure at 12 months. Secondary endpoints included cardiac death, hospitalization for heart failure, major cardiovascular adverse events (MACE; a composite of all-cause death, reinfarction, coronary revascularization, and stroke), myocardial infarction, stroke, revascularization, and all-cause death. A blinded independent endpoint committee reviewed all events. A detailed description of endpoint definitions has been published elsewhere [29].

Statistical analysis

Patients were stratified according to peri-procedural treatment with clopidogrel, ticagrelor or prasugrel. We used propensity score based-methods to estimate the average treatment effect of ticagrelor or prasugrel compared to clopidogrel [55].

For the infarct size calculations, we estimated 48-hour troponin T AUC for subsets of patients using multiple imputation by chained equations in case of missing data. We log-transformed AUC since distributions were skewed, and computed the AUC ratio by linear regression. AUC ratios were calculated in the propensity score cohorts characterized below. In the main analysis, we compared clopidogrel vs. ticagrelor vs. prasugrel in a combined analysis. For the sensitivity analyses, comparisons between clopidogrel vs. ticagrelor and clopidogrel vs. prasugrel were analyzed separately because the number of patients were higher than in the combined analysis.

Covariates associated with both the outcome and exposure or only the outcome were included to estimate the propensity score: age (continuous variable), sex, body mass index (<18.5 kg/m^2, 18.5-24.9 kg/m^2, 25-29.9 kg/m^2, ≥30 kg/m^2), active smoking, hypertension, previous myocardial infarction, hypercholesterolemia, diabetes, first medical contact to balloon time (<60 minutes, 60-119 minutes, 120-179 minutes, ≥180 minutes) [59], multivessel disease, LAD stenosis, Killip class,

Thrombolysis In Myocardial Infarction Flow Grade, periprocedural heparin, and country [15]. The original CONDI-2/ERIC-PPCI trial analyses showed no interaction between treatment with ticagrelor and RIC [29]. A total of 17.3% of patients had missing values in ≥1 of the covariates included in the propensity score. Missing values were handled through multiple imputations using chained equations, generating 20 imputations. We used multinomial logistic regression to estimate the propensity of type of $P2Y_{12}$ receptor inhibitor. A Cox regression was used to estimate crude and stabilized inverse-probability-weighted (IPW) hazard ratios (HRs) using clopidogrel as reference [16, 30]. The proportional hazards assumption was evaluated by log–log plots, and found to be satisfied. Twelve-month cumulative incidence proportion was estimated, accounting for the competing risk of all-cause death, except in the case of MACE and all-cause death. Twelve-month cumulative incidence curves of the main outcome and MACE were constructed. We also estimated the 30-day risk of the main outcome and MACE.

We performed two sensitivity analyses. First, a 'full cohort' analysis in which all patients received ticagrelor, prasugrel or clopidogrel in relation to PPCI, including patients who were not eligible in propensity score based-analyses. We estimated adjusted HRs by multivariable Cox regression. We adjusted for the same covariates used for the propensity score. Second, a propensity-score based analysis in which we analyzed the data in two separate analyses, one comparing clopidogrel and ticagrelor, and one comparing clopidogrel and prasugrel. In the separated analyses all Spanish patients were excluded due to structural non-positivity, since all Spanish patients were treated with clopidogrel [30]. For the same reason all patients from Serbia were excluded from the analysis of prasugrel vs clopidogrel, since no patients in Serbia received prasugrel. To improve balance in distribution of propensity scores in the treatment groups, patients with a propensity score <0.1 and >0.9 were excluded [20].

All statistical analyses of clinical data were performed using StataIC version 16 (Stata Corp, College Station, Texas, USA).

Results

Animal experiments – Infarct size

IPC significantly reduced infarct size compared to controls (26±12% vs 52±8%, $p<0.0001$) (Figure 1). RIC also reduced infarct size compared to controls, but not to the same degree as IPC (41±11 vs 52±8%, $p<0.05$).

Ticagrelor reduced infarct size compared to controls (37±11% vs 52±8%, $p<0.01$). Clopidogrel or prasugrel did not affect infarct size (50±11%, $p>0.99$ and 49±9%, $p>0.99$, respectively).

Combination therapy with IPC and ticagrelor resulted in a reduction in infarct size compared to controls (25±9% vs 52±8%, $p<0.0001$). The reduction in infarct size was similar to IPC treatment alone ($p>0.99$), suggesting no additive cardioprotective effect with the combination of IPC and ticagrelor.

The reduction in infarct size from combination therapy with RIC and ticagrelor was similar treatment with RIC alone (42±13, $p>0.99$), but the reduction only reached borderline statistical significance when compared to controls ($p=0.08$). Again, there was no additive cardioprotective effect with the combination of RIC and ticagrelor.

Interaction analyses of infarct size showed interaction between ticagrelor treatment and IPC ($p<0.05$) and RIC ($p<0.05$) (Table S1).

Infarct size related to left ventricle showed the same results as infarct size related to area at risk. With an average of 40% of left ventricle, area at risk did not differ between any of the intervention groups and controls.

Clinical study – infarct size

In our combined analysis, the 48-hour AUC of troponin release was reduced in patients treated with ticagrelor compared to clopidogrel (Adjusted AUC ratio: 0.67, 95% CI 0.47-0.94) (Table S2). The number of prasugrel treated patients with troponin data (n=5) did not allow sufficient statistical power to provide valid results (Table S2). The supplementary sensitivity analysis, where AUC troponin release was compared separately as clopidogrel vs. ticagrelor and clopidogrel vs. prasugrel, showed no significant reduction in troponin release from either ticagrelor or prasugrel (Table S3).

Clinical study - outcome

Out of 5115 patients included in the original CONDI-2/ERIC-PPCI study, we included a total of 1754 patients in the retrospective main analysis (Figure 2). Of these 395 patients received clopidogrel, 1210 patients received ticagrelor and 149 received prasugrel. The number of patients differs between the groups, as patient are included based on the propensity scores. Baseline characteristics of the patients included in the analysis are shown in table 1. All three $P2Y_{12}$ inhibitors were only prescribed in the UK. Thus, only UK patients were ultimately included in the multinominal logistic regression analysis. Baseline characteristics were generally well balanced. Patients with previous myocardial infarction were slightly more prevalent in the groups treated with ticagrelor (37.6%) and prasugrel (41.6%) compared to clopidogrel (25.6%). Nitrates were used more often in patients treated with clopidogrel (89.4%) and prasugrel (92.6%) compared to ticagrelor (81.3%), which may be due to regional differences in medication strategy.

The main composite outcome of one-year risk of cardiac death or hospitalization for heart failure occurred in 9.6% of the clopidogrel treated patients, compared to 6.5% in the ticagrelor treated patients (HR 0.63; 95% CI 0.42-0.94) and 8.1% in the prasugrel treated patients (HR 0.84,

95% CI 0.43-1.63) (Table 2) with the time course specified in Figure 3. In analyses of the individual components of the composite primary endpoint ticagrelor reduced the risk to a similar extent, but not all with statistical significance: one-year risk of hospitalization for heart failure (HR 0.57, 95% CI 0.36-0.91), cardiac death (HR 0.78, 95% CI 0.38-1.58) and all-cause death (HR 0.59, 95% CI 0.35-1.00) (Table S4). The individual components of the primary endpoint were not affected by prasugrel: one-year risk of hospitalization for heart failure (HR 0.98, 95% CI 0.48-1.99), cardiac death (HR 0.28, 95% CI 0.04-2.19) and all-cause death (HR 0.24, 95% CI 0.06-1.04) (Table S4). The one-year risk of MACE was not affected by ticagrelor (HR 0.86, 95% CI 0.56-1.32) or prasugrel (HR 0.54, 95% CI 0.24-1.23) compared to clopidogrel (Table 2 and Figure 3). Reinfarction, stroke or revascularization did not differ between groups (Table S4).

The thirty-day risk of cardiac death or hospitalization for heart failure was reduced in patients receiving ticagrelor (HR 0.64, 95% CI 0.42-0.98), but not prasugrel (HR 0.80, 95% CI 0.39-1.65) compared to clopidogrel (Figure 3 and Table S5). No reduction in MACE was found at 30 days.

The sensitivity analyses of the composite clinical endpoint yielded results consistent with the main analysis (Table S6-S10, Figure S2 and S3).

Discussion

The results of our study demonstrate that ticagrelor but not clopidogrel or prasugrel decreased infarct size in an *in vivo* rat model of ischemia reperfusion injury. Correspondingly, ticagrelor seemed to reduce infarct size as measured by troponin release in the clinical setting in a post-hoc sub-study. The results translated into a beneficial effect of ticagrelor pre-treatment in terms of a reduced incidence of a composite endpoint including cardiac death and hospitalization for heart failure with contribution of each component.

Effect of $P2Y_{12}$ inhibitors on infarct size

Beyond the documented beneficial antithrombotic effects on myocardial damage [19, 41, 42, 50, 53, 67, 70, 78], experimental studies have shown that second and third generation $P2Y_{12}$ inhibitors may be capable of reducing infarct size in experimental settings, but their cardioprotective capacity appears variable [9, 25, 66, 72, 74, 76]. Translation into a potential clinical effect was already demonstrated for the second generation $P2Y_{12}$ inhibitor, clopidogrel, but appears to vary as well [24, 51].

Our results confirm that ticagrelor reduces infarct size in experimental models of ischemia-reperfusion injury [9, 22, 48, 79]. Cardioprotection can be obtained by a single dose given only two hours before myocardial infarction [3, 72, 76], which potentially increases clinical translation because cangrelor, an intravenously administered equivalent to ticagrelor, has cardioprotective effects when given just before reperfusion [75]. The mechanisms behind the ticagrelor-induced cardioprotection seem not solely related to the inhibition of platelet aggregation, but also to pleiotropic effects [22].

Among the orally administered $P2Y_{12}$ inhibitors, ticagrelor has most convincingly demonstrated infarct size reduction in STEMI patients undergoing rapid revascularization using

measurement of troponin release [47, 52] and more reliably by magnetic resonance imaging [37, 52]. Although our statistical analyses are not completely consistent due to our retrospective design and suboptimal statistical power, our data do not dispute that the beneficial clinical outcome with ticagrelor was associated with reduced infarct size, measured by troponin release in patients. We acknowledge that the measurement of infarct size by circulating biomarkers should be interpreted with caution. Our main troponin analysis relies on 260 patients. Only 5 patients in the analyses received prasugrel, such that a valid estimate was not obtainable. The sensitivity analyses of troponin release in a pairwise comparison between clopidogrel and ticagrelor in 503 patients did not confirm the results of the main analysis. When infarct sizes are minor, i.e. in the order of magnitude of 16% of the left ventricle, as obtained by modern reperfusion therapy [14], the sensitivity of circulating biomarkers may not be optimal.

Experimental studies of the cardioprotective effect of pretreatment with prasugrel are limited and with varying results [9, 25, 44]. Despite three days of pretreatment with prasugrel, Birnbaum *et al* did not show infarct reduction after coronary occlusion [9], whereas Dost *et al* demonstrated that a single dose prasugrel reduced infarct size [25]. We found no reduction in infarct size by a single dose of prasugrel, although our experimental setup seemed similar to the approach used by Dost *et al.* in terms of dosing, timing and ischemia/reperfusion protocol. The use of two different rat strains may explain the discrepancy as sensitivity to ischemia and reperfusion injury is known to vary between rat strains [5].

Effect of $P2Y_{12}$ inhibitors on clinical outcome

In accordance with The Ticagrelor Therapy in STEMI Patients Planned for Percutanous Coronary Intervention (ATLANTIC) trial [45], we observed no reduction in MACE, potentially reflecting that the benefit of ticagrelor is not caused only by a more efficient long-term platelet

inhibition than with clopidogrel. A statistically significant improvement by prasugrel compared to clopidogrel treatment was not evident from our main endpoint. Consistent with our results a prespecified substudy of the ISAR REACT 5 trial [53] in STEMI-patients demonstrated no significant difference in the primary endpoint (incidence of death, myocardial infarction, or stroke at 1 year after randomization) between prasugrel and ticagrelor [4]. The endpoint in the ISAR REACT 5 study mainly relates to the antithrombotic effect of the $P2Y_{12}$ inhibitors. We observed a reduction of cardiac death or hospitalization for heart failure by ticagrelor that emerged early compared to clopidogrel but compared to prasugrel most clearly after 180 days of follow-up (Figure 3a and b). The early effect may reflect a superior antithrombotic efficacy of prasugrel and ticagrelor compared to clopidogrel, whereas infarct size reduction by ticagrelor becomes evident with a delay when inappropriate remodeling due to a significant MI size translates into clinal symptoms.

Mechanistic considerations

In observational post hoc analyses, the effect of the third generation $P2Y_{12}$ inhibitors, ticagrelor and prasugrel, versus the second-generation inhibitor, clopidogrel, on microvascular obstruction is equivocal [36, 65]. Ticagrelor does not seem to be superior to prasugrel in reducing microvascular obstruction [62, 64]. Although experimental data suggest that $P2Y_{12}$-receptor inhibition using cangrelor at the onset of reperfusion can itself reduce MI size [75], it is unclear whether the cardioprotective effect is mediated on the coronary vasculature or the cardiomyocyte [28, 36, 37]. Ticagrelor increases circulating levels of adenosine in humans mainly at doses higher than standard [61]. Still, increased serum concentration of adenosine seems to be responsible for ticagrelor-related adverse effects, including dyspnea, ventricular pauses, and bradyarrhythmias. Moreover, experimental as well as human studies suggest that ticagrelor enhances the biological effects of endogenous adenosine [63, 69], implying that adenosine may serve as a mediator of some

of the pleiotropic cardioprotective effect [1, 61, 69]. Ticagrelor has a favorable effect on endothelial function after ischemia and reperfusion compared to clopidogrel in humans [68]. The A_{2A} receptor is the main adenosine receptor responsible for coronary vasodilation, mediated by both nitric oxide-dependent and -independent pathways [46]. Adenosine may also act cardioprotective by inhibiting neutrophil trafficking, granule release, and production of reactive oxygen species and inflammatory mediators [6, 21, 49].

Studies of cardioprotection by $P2Y_{12}$ inhibitors imply that activated platelets are involved although interference with platelet aggregation itself [18, 48, 73] or improved early coronary reperfusion [24] may not be the main targets. Despite faster $P2Y_{12}$ inhibition by cangrelor, compared to ticagrelor, this does not necessarily induce an increased salvage of myocardium [60]. Near-obliteration of circulating platelets either with cell poison or an antibody abrogates the cardioprotective effect of $P2Y_{12}$ antagonists [18]. Furthermore, $P2Y_{12}$ antagonists have no effect in isolated hearts perfused with platelet-free buffer [18, 73]. Platelets may be a target of $P2Y_{12}$ antagonists for creation of a cardioprotective effect [7]. However, it is unknown whether activated platelets release substances with protective effects on the endothelium and how events between binding of the $P2Y_{12}$ blocker to its platelet receptor relates to emergence of cardioprotection.

Platelet reactivity declines relatively slowly after oral administration of $P2Y_{12}$ inhibitors and requires several hours before reaching full effect. The profile is most favorable for oral ticagrelor or prasugrel administration as manifestation of the antiplatelet activity within 1-3 hours [2, 8, 10, 26] is less than for clopidogrel for which the effect initiates after 6-11 hours [8]. It remains unknown whether the timing of $P2Y_{12}$ inhibitors in the CONDI2-PPCI trial was optimal. In the original trial we only had access to data on the type of $P2Y_{12}$ inhibitor treatment given in relation to the PPCI and not the timing of the administration or the treatment strategy after the index event. Nonetheless, ticagrelor improved the main endpoint indicating that the effect was sufficient.

Interaction of $P2Y_{12}$ inhibitor treatment and ischemic conditioning

In preclinical studies, ischemic pre- and postconditioning conditioning interact with the cardioprotective effect of the $P2Y_{12}$ inhibitor cangrelor, with no additional effect of combination therapy of $P2Y_{12}$ inhibition and ischemic conditioning [72, 74]. We found a similar interaction with no additive effect between ticagrelor treatment and both IPC and RIC in our experimental data and extended the knowledge of an interaction between ischemic conditioning and $P2Y_{12}$ inhibitors to include RIC. Combination treatment with ticagrelor and RIC did not significantly reduce infarct size. Whether this is solely related to the larger variation in the data or whether other factors are responsible is not known. In our clinical trial, we observed no interaction between RIC and ticagrelor treatment due to the lack of effect by RIC on the main endpoints [29]. Eventually, a potential interaction may be explained by two different even opposing mechanisms, which are almost undifferentiable: 1. There is a potential recruitment of protection by the patient medications and 2. Protection can be attenuated/abrogated by the medication and in parallel [38]. These two mechanisms may interfere in different ways with IPC and RIC, since the underlying signal transduction of IPC and RIC may also differ [32, 39]. In an experimental setting, IPC improved the recovery of coronary flow and LVDP during reperfusion whereas RIC only impacted on the recovery of LVDP [40], indicating that IPC may exert stronger protective effects on the coronary vasculature than RIC [31]. While the near maximum efficacy of IPC and RIC seems to have been reached in our experimental setting, a further protection potential by an intensified stimulus [35, 40, 54] may be present in the clinical setting [71]. Deployment of the full protection capacity seems to be necessary to uncover an interaction with pharmacological treatment and should be taken into consideration when applying multitarget strategies.

Clinical implications

The signal indicating that ticagrelor has the most potent cardioprotective capacity among currently recommended oral $P2Y_{12}$ inhibitors seems to contribute to the improvement in clinical outcome by modern standard care of patients with STEMI. The achieved improvement in clinical outcome may challenge the ability to document adjunctive cardioprotective treatments beyond optimized standard care in future studies. We have realized this challenge when changing from clopidogrel to ticagrelor in our previous clinical studies of RIC [14, 29]. Careful selection of high-risk patients [11, 12] and multitarget cardioprotective strategies may increase the protective potential [23, 33]. Since intravenous cangrelor may have similar cardioprotective efficacy as ticagrelor, an alternative treatment strategy might be intravenous cangrelor infusion shortly prior to stenting followed by subsequent post-PCI transition to an oral agent [17].

Study limitations

To investigate the cardioprotective capacity of $P2Y_{12}$ inhibitors we chose an *in vivo* rat model, as the $P2Y_{12}$ inhibitors necessitate *in vivo* metabolization of the drugs and presence of platelets. Since IPC was induced by an invasive procedure, the experimental design was limited by the prolonged surgical procedure in these groups. Our unpublished pilot trials showed no impact on infarct size from a prolonged surgical procedure in control animals. As IPC and RIC may have different signaling profiles, we cannot exclude an effect of the timing between IPC or RIC and index ischemia. Doses of $P2Y_{12}$ inhibitors relied on results from other laboratories [25, 44, 66, 72, 74, 76], so we did not conduct dose-response experiments and it cannot be excluded that other doses of $P2Y_{12}$ inhibitors might increase the cardioprotective effects.

The main limitation of the clinical part of the study is that the patients in the original CONDI-2/ERIC-PPCI trial were not randomized by $P2Y_{12}$ inhibitor prescription. To reduce confounding, our statistical analyses rely on propensity scored analyses. To balance the distribution

of propensity score between treatment groups, we excluded both low and high propensity patients, hereby reducing the sample size. Also, relatively few patients in our cohort were treated with prasugrel, so estimates of clinical outcome in prasugrel treated patients are with higher statistical uncertainty. The sensitivity analyses with pairwise comparison of ticagrelor and clopidogrel and ticagrelor and prasugrel had larger cohorts. Consequently, the statistical power was increased. The sensitivity analyses confirmed the results of the main analysis for the primary clinical outcome but not infarct size data. Moreover, residual confounding may still be present, so our results should be considered exploratory and needs confirmation in a randomized trial.

Conclusion

Pre-treatment with ticagrelor reduced infarct size in rats after ischemia and reperfusion injury, whereas clopidogrel or prasugrel did not. In patients suffering from STEMI and treated with PPCI, we found that treatment with ticagrelor, but not prasugrel, reduced cardiac death and hospitalization for heart failure compared to treatment with clopidogrel. The improved clinical outcome with ticagrelor may be caused by pleiotropic effects that attenuate ischemia and reperfusion injury.

Declarations

Funding Sources

The ERIC-PPCI trial was funded by a British Heart Foundation Clinical Study Grant (CS/14/3/31002) and a University College London Hospital/University College London Biomedical Research Clinical Research grant. The CONDI-2 trial was funded by Danish Innovation Foundation grants (11-108354 and 11-115818), Novo Nordisk Foundation (NNF13OC0007447), and Trygfonden (109624). DJH was supported by the British Heart Foundation (FS/10/039/28270), Duke-National University Singapore Medical School, Singapore Ministry of Health's National Medical Research Council under its Clinician Scientist-Senior Investigator scheme (NMRC/CSA-SI/0011/2017) and its Collaborative Centre Grant scheme (NMRC/CGAug16C006). This article is based upon the work of COST Action EU-CARDIOPROTECTION (CA16225) and supported by COST (European Cooperation in Science and Technology).

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

The ethical approval of the animal experiments of the study was approved by the Danish Veterinary and Food Administration (Authorization number: 2018-15-0201-01475). The clinical part of the study was authorized on ClinicalTrials.gov as NCT02342522.

Availability of data and material

Data can be made available if the manuscript is accepted for publication.

Code availability

Not applicable.

Author contributions

All authors have participated in parts of the conception and design of the study or analysis and interpretation of data.

Authors responsible for the animal experiments: MV Hjortbak, JM Seefeldt, TR Lassen, RV Jensen and HE Bøtker.

Authors responsible for the clinical analyses: KKW Olesen, MV Hjortbak, A Perkins, M Dodd, T Clayton, D Yellon, DJ Hausenloy and HE Bøtker.

All authors have contributed to drafting the manuscript, and all have read and approved the manuscript.

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Figure titles and legends

Figure 1 Infarct size in rats. Final infarct size of area at risk. IS: infarct size, AAR: area at risk, CON: control, IPC: local ischemic preconditioning, RIC: remote ischemic preconditioning. All statistical comparisons showed have controls as reference. * $p<0.05$, ** $p<0.01$, **** $p<0.0001$

Figure 2 Flowchart. Flowchart of patient selection and exclusions.

Figure 3 Graphical presentations. a) the composite endpoint of cardiac death and b) hospitalization for heart failure, and major adverse cardiovascular events, c) and d) display 30-day curves of the same endpoints.

Tables

Table 1. Baseline characteristics and procedural details.

	Clopidogrel	**Ticagrelor**	**Prasugrel**
	(n=395)	(n=1,210)	(n=149)
Mean age, years (SD)	65.7 (12.4)	63.7 (12.0)	61.1 (11.0)
Sex			
Male	294 (74.4%)	946 (78.2%)	125 (83.9%)
Female	101 (25.6%)	264 (21.8%)	24 (16.1%)
Current smoker	132 (33.4%)	437 (36.1%)	59 (39.6%)
Body mass index, kg/m^2 (SD)	27.5 (4.8)	27.6 (5.1)	28.2 (4.5)
eGFR, μg/L/1.73 m^2 (IQR)	87 (71-97)	86 (72-96)	87 (77-99)
Comorbidity			
Hypertension	175 (44.3%)	489 (40.4%)	58 (38.9%)
Previous MI	35 (8.9%)	95 (7.9%)	10 (6.7%)
Hypercholesterolaemia	121 (30.6%)	353 (29.2%)	46 (30.9%)
Peripheral vascular disease	9 (2.3%)	18 (1.5%)	1 (0.7%)
Diabetes	48 (12.2%)	131 (10.8%)	15 (10.1%)
Family history of IHD	101 (25.6%)	455 (37.6%)	62 (41.6%)
Baseline medication			
Insulin	12 (3%)	31 (2.6%)	5 (3.4%)
Metformin	39 (9.9%)	100 (8.3%)	10 (6.7%)
Sulphonylurea	14 (3.5%)	37 (3.1%)	5 (3.4%)
Other anti-diabetic medication	16 (4.1%)	29 (2.4%)	5 (3.4%)
Statin	98 (24.8%)	272 (22.5%)	35 (23.5%)
Beta-blocker	49 (12.4%)	139 (11.5%)	18 (12.1%)
ACE-inhibitor	74 (18.7%)	194 (16%)	31 (20.8%)
ARB	40 (10.1%)	106 (8.8%)	9 (6%)

Aspirin	53 (13.4%)	180 (14.9%)	15 (10.1%)
Diuretics	32 (8.1%)	85 (7%)	18 (12.1%)
Blood pressure at inclusion (mmHg)			
Systolic (SD)	132.1 (25.9)	133.1 (22.7)	126.3 (22.6)
Diastolic (SD)	78.7 (16.4)	80.2 (15.7)	73.6 (15.0)
Killip Class on admission			
Class I	389 (98.5%)	1,172 (96.9%)	145 (97.3%)
Class II	2 (0.5%)	33 (2.7%)	1 (0.7%)
Class III	0	1 (0.1%)	0
Class IV (including cardiogenic shock)	4 (1%)	4 (0.3%)	3 (2%)
Symptom to balloon time, min (IQR)	177 (129-261)	185 (138-298)	176 (121-257)
First medical contact to balloon time, min (IQR)	105 (90-127)	112 (92-137)	104 (86-132)
Culprit vessel			
Left anterior descending	160 (40.5%)	489 (40.4%)	62 (41.6%)
Circumflex	43 (10.9%)	160 (13.2%)	21 (14.1%)
Right coronary	190 (48.1%)	551 (45.5%)	63 (42.3%)
Other	0	4 (0.3%)	0
Missing	2 (0.5%)	6 (0.5%)	3 (2%)
Culprit lesion stented	367 (92.9%)	1,170 (96.7%)	139 (93.3%)
Number of vessels with angiographically significant disease			
0	0	2 (0.2%)	0
1	222 (56.2%)	639 (52.8%)	89 (59.7%)
2	125 (31.6%)	388 (32.1%)	39 (26.2%)
3	46 (11.6%)	176 (14.5%)	18 (12.1%)
Missing	2 (0.5%)	5 (0.4%)	3 (2%)
Thrombus aspiration performed	115 (29.1%)	378 (31.2%)	39 (26.2%)
TIMI flow pre-angioplasty			
TIMI 0	284 (71.9%)	944 (78%)	116 (77.9%)

TIMI 1	31 (7.8%)	72 (6%)	12 (8.1%)
TIMI 2	32 (8.1%)	88 (7.3%)	12 (8.1%)
TIMI 3	48 (12.2%)	106 (8.8%)	9 (6%)
Missing	284 (71.9%)	944 (78%)	116 (77.9%)
TIMI flow post-procedure			
TIMI 0	5 (1.3%)	14 (1.2%)	0
TIMI 1	4 (1%)	7 (0.6%)	1 (0.7%)
TIMI 2	27 (6.8%)	56 (4.6%)	5 (3.4%)
TIMI 3	351 (88.9%)	1,092 (90.2%)	139 (93.3%)
Missing	8 (2%)	41 (3.4%)	4 (2.7%)
Staged PCI performed	36 (9.1%)	108 (8.9%)	7 (4.7%)
Staged CABG performed	5 (1.3%)	21 (1.7%)	2 (1.3%)
pPCI related medication			
Opioids	0	0	0
Heparin	372 (94.2%)	1,159 (95.8%)	144 (96.6%)
Aspirin	375 (94.9%)	1,136 (93.9%)	138 (92.6%)
Glycoprotein IIb/IIIa inhibitor	94 (23.8%)	324 (26.8%)	45 (30.2%)
Bivalirudin	21 (5.3%)	35 (2.9%)	0
Protaminsulphate	2 (0.5%)	5 (0.4%)	1 (0.7%)
Nitrates	353 (89.4%)	984 (81.3%)	138 (92.6%)
Country			
UK	395 (100%)	1,210 (100%)	149 (100%)

Table 1. Baseline characteristics of the patient cohorts included in the propensity weighted analyses. On the left the cohorts included in the analyses of clopidogrel vs ticagrelor, and on the right the cohorts included in the analyses of clopidogrel vs prasugrel. IHD: Ischemic heart disease, ACE: Angiotensin converting enzyme, ARB: Angiotensin receptor blocker, TIMI: Thrombolysis in myocardial infarction, SD: Standard deviation, IQR: Interquartile range.

Table 2. One-year cardiovascular risk in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention treated with either clopidogrel, ticagrelor, or prasugrel.

	Patients	Events	Cumulative incidence proportion (95% CI)	Unadjusted HR (95% CI)	Stabilized IPW weighted HR (95% CI)
Cardiac death or hospitalization for heart failure					
Clopidogrel	395	38	9.6% (7.0-12.8)	reference	reference
Ticagrelor	1,210	78	6.5% (5.2-7.9)	0.66 (0.45-0.97)	0.63 (0.42-0.94)
Prasugrel	149	12	8.1% (4.4-13.1)	0.83 (0.44-1.57)	0.84 (0.43-1.63)
Major adverse cardiovascular events					
Clopidogrel	395	30	7.6% (5.4-10.7)	reference	reference
Ticagrelor	1,210	83	6.9% (5.6-8.4)	0.90 (0.59-1.36)	0.86 (0.56-1.32)
Prasugrel	149	8	5.4% (2.7-10.5)	0.70 (0.32-1.53)	0.54 (0.24-1.23)

Table 2 One-year cardiovascular risk. One-year risk of cardiac death or hospitalization for heart failure, or major adverse cardiovascular events (MACE) in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention treated with either ticagrelor compared to clopidogrel, or prasugrel compared to clopidogrel. HR: hazard ratios, IPW: inverse-probability-weighted.