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2 **Translation of experimental cardioprotective capability of P2Y₁₂**
3 **inhibitors into clinical outcome in patients with ST-elevation**
4 **myocardial infarction**

5
6 Short title: Clinical impact of P2Y₁₂ inhibitors' cardioprotective capability

7
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1 **Abstract**

2 **Objectives:** We studied the translational cardioprotective potential of P2Y₁₂ inhibitors against acute
3 myocardial ischemia/reperfusion injury (IRI) in an animal model of acute myocardial infarction and
4 in patients with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous
5 coronary intervention (PPCI).

6 **Background:** P2Y₁₂ inhibitors have pleiotropic effects that may induce cardioprotection against
7 acute myocardial IRI beyond their inhibitory effects on platelet aggregation.

8 **Methods:** We compared the cardioprotective effects of clopidogrel, prasugrel and ticagrelor on
9 infarct size in an *in vivo* rat model of acute myocardial IRI, and investigated the effects of the P2Y₁₂
10 inhibitors on enzymatic infarct size (48-hour area-under-the-curve (AUC) troponin T release) and
11 clinical outcomes in a retrospective study of STEMI patients from the CONDI-2/ERIC-PPCI trial
12 using propensity score analyses.

13 **Results:** Loading with ticagrelor in rats reduced infarct size after acute myocardial IRI compared to
14 controls (37±11% vs 52±8%, p<0.01), whereas clopidogrel and prasugrel did not (50±11%, p>0.99
15 and 49±9%, p>0.99, respectively). Correspondingly, troponin release was reduced in STEMI patients
16 treated with ticagrelor compared to clopidogrel (adjusted 48-hour AUC ratio: 0.67, 95% CI 0.47-
17 0.94). Compared to clopidogrel the composite endpoint of cardiac death or hospitalization for heart
18 failure within 12 months was reduced in STEMI patients loaded with ticagrelor (HR 0.63; 95% CI
19 0.42-0.94) but not prasugrel (HR 0.84, 95% CI 0.43-1.63), prior to PPCI. Major adverse
20 cardiovascular events did not differ between clopidogrel, ticagrelor or prasugrel.

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

23

24 **Key words:** P2Y₁₂ inhibitor, cardioprotection, ischemic conditioning, myocardial infarction

1 **Introduction**

2 Acute myocardial infarction still contributes to mortality and morbidity worldwide. During
3 myocardial infarction, the myocardium suffers ischemic damage, which can only be targeted by
4 timely reperfusion therapy. The paradoxical myocardial reperfusion injury that may extend final
5 infarct size [77] requires adjunctive treatment strategies beyond reperfusion to improve clinical
6 outcome. Although remote ischemic conditioning (RIC) reduces myocardial injury by activating
7 inherent cardioprotective mechanisms [34], verification of a clinical benefit for the patients has been
8 challenging, mainly because clinical event rates with modern reperfusion therapy are low [27, 29,
9 43].

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

15 Loading treatment with P2Y₁₂ inhibitors is an established adjunctive therapy to invasive
16 treatment of acute coronary syndrome because of their inhibitory effect on platelet aggregation.
17 However, clopidogrel, prasugrel and ticagrelor have all demonstrated pleiotropic, cardioprotective
18 effects in experimental studies [72, 74]. Observations from minor, retrospective studies indicate that
19 the cardioprotective effects of P2Y₁₂ inhibitors may be transferrable to a clinical setting [36, 52].

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

24

1 **Methods**

2 **Rat experiments**

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

9

10 *Delivery of P2Y₁₂ inhibitors*

11 P2Y₁₂ inhibitors were administered by oral gavage using crushed tablets suspended in tab water; doses
12 were adjusted to body weight of the individual rat. Clopidogrel (15 mg/kg) (Clopidogrel STADA,
13 STADA Arzneimittel AG, Bad Vilbel, Germany) was given 4 hours prior to induction of myocardial
14 ischemia, ticagrelor (20 mg/kg)(Brilique, AstraZeneca, Cambridge, United Kingdom) and prasugrel
15 (10 mg/kg) (Efient, Daiichi-Sankyo Europe GmbH, Munich, Germany) were given 2 hours prior to
16 induction of myocardial ischemia. Placebo treatment consisted of tab water only given 2 hours before
17 myocardial ischemia.

18 The dosage and timing of P2Y₁₂ inhibitors were chosen from available data in the
19 literature. Clopidogrel is a prodrug that requires enzymatic activation. The loading dose of
20 clopidogrel must be given before reperfusion of the myocardium, but the duration of pretreatment to
21 induce protection varies between 4 hours and two days in animal studies [66, 74]. In the present study
22 we loaded the animals with clopidogrel 4 hours prior to induction of ischemia because the resultant
23 plasma concentration is associated with antiplatelet efficacy [56, 66] and because the approach may
24 have some potential for clinical translation when given before reperfusion. The dose of clopidogrel

1 was based on previous studies demonstrating cardioprotective effect of clopidogrel [66, 76].
2 Ticagrelor and prasugrel have more rapid and potent antiplatelet responses than clopidogrel. In rats,
3 platelet aggregation is significantly inhibited one to two hours after administration of ticagrelor or
4 prasugrel, whereas clopidogrel may require 2-4 hours [56, 57]. This pharmacologic profile may
5 increase the cardioprotective potential of ticagrelor and prasugrel within a clinically relevant
6 timeframe for STEMI patients. As for clopidogrel, the doses of prasugrel [25, 58] and ticagrelor [3,
7 66, 72, 76] were based on previous studies demonstrating cardioprotective effect.

8

9 *In vivo myocardial infarction*

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

18 RIC was performed prior to the thoracotomy using a tourniquet around a hind leg, to induce
19 3 cycles of 5 minutes limb ischemia followed by 5 minutes of reperfusion. IPC was performed after
20 the thoracotomy, using the myocardial suture around LAD to induce 3 cycles of 5 minutes of ischemia
21 followed by 5 minutes of reperfusion.

22

23

24

1 *Infarct size*

2 After 2 hours of reperfusion, the LAD was reoccluded, and a 2 % solution of Evans Blue (Sigma-
3 Aldrich, St. Louis, MO, USA) was injected in the inferior vena cava to visualize the area at risk. The
4 hearts were rapidly removed and stored at -80 °C. The hearts were then sliced and stained using a 1%
5 solution of Triphenyl Tetrazolium Chloride (Sigma-Aldrich, St Louis, MO, USA). After 24 hours in
6 4% formalin buffer (VWR International, Leuven, Belgium), the slices were scanned using a flatbed
7 scanner (Epson Perfection V600 Photo scanner, Epson, Nagano, Japan). The infarct size, area at risk
8 and area of the left ventricle were assessed using ImageJ software (NIH, Bethesda, Maryland, USA).
9 All measurements were correlated to the wet weight of the individual slice. Final infarct size is
10 expressed as the percent of infarcted area over the area at risk.

11

12 *Statistical analyses*

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

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

22

23

24

1 **Clinical studies**

2 The clinical part of the study was designed as a retrospective, non-prespecified post hoc sub-study of
3 the international, multicenter, single-blind, randomized controlled CONDI-2/ERIC-PPCI trial [29].
4 A detailed description of the study is provided in the original publication [29]. Patients with ST-
5 segment elevation myocardial infarction, eligible to PPCI, were randomized to standard treatment or
6 treatment with RIC. The study included patients from 33 centers across United Kingdom, Denmark,
7 Spain and Serbia. We analyzed the data collected for the CONDI-2/ERIC-PPC trial to investigate
8 interaction between treatment with P2Y₁₂ receptor inhibitors and RIC in relation to PPCI for clinical
9 outcomes.

10 In accordance with contemporary guidelines, patients with STEMI were loaded with a
11 P2Y₁₂ receptor inhibitor prior to PPCI. Patients received either clopidogrel (600mg), ticagrelor (180
12 mg) or prasugrel (60 mg). Choice of P2Y₁₂ receptor inhibitor for loading was based on current
13 guidelines and regional preferences. The time from administration of the chosen P2Y₁₂ inhibitor to
14 reperfusion by PPCI was not registered.

15

16 *Patient Selection*

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

20

21 *Infarct size*

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

24

1 *Clinical outcomes*

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

8
9 *Statistical analysis*

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

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

20 Covariates associated with both the outcome and exposure or only the outcome were included
21 to estimate the propensity score: age (continuous variable), sex, body mass index (<18.5 kg/m², 18.5-
22 24.9 kg/m², 25-29.9 kg/m², ≥30 kg/m²), active smoking, hypertension, previous myocardial
23 infarction, hypercholesterolemia, diabetes, first medical contact to balloon time (<60 minutes, 60-
24 119 minutes, 120-179 minutes, ≥180 minutes) [59], multivessel disease, LAD stenosis, Killip class,

1 Thrombolysis In Myocardial Infarction Flow Grade, periprocedural heparin, and country [15]. The
2 original CONDI-2/ERIC-PPCI trial analyses showed no interaction between treatment with ticagrelor
3 and RIC [29]. A total of 17.3% of patients had missing values in ≥ 1 of the covariates included in the
4 propensity score. Missing values were handled through multiple imputations using chained equations,
5 generating 20 imputations. We used multinomial logistic regression to estimate the propensity of type
6 of P2Y₁₂ receptor inhibitor. A Cox regression was used to estimate crude and stabilized inverse-
7 probability-weighted (IPW) hazard ratios (HRs) using clopidogrel as reference [16, 30]. The
8 proportional hazards assumption was evaluated by log–log plots, and found to be satisfied. Twelve-
9 month cumulative incidence proportion was estimated, accounting for the competing risk of all-cause
10 death, except in the case of MACE and all-cause death. Twelve-month cumulative incidence curves
11 of the main outcome and MACE were constructed. We also estimated the 30-day risk of the main
12 outcome and MACE.

13 We performed two sensitivity analyses. First, a ‘full cohort’ analysis in which all patients
14 received ticagrelor, prasugrel or clopidogrel in relation to PPCI, including patients who were not
15 eligible in propensity score based-analyses. We estimated adjusted HRs by multivariable Cox
16 regression. We adjusted for the same covariates used for the propensity score. Second, a propensity-
17 score based analysis in which we analyzed the data in two separate analyses, one comparing
18 clopidogrel and ticagrelor, and one comparing clopidogrel and prasugrel. In the separated analyses
19 all Spanish patients were excluded due to structural non-positivity, since all Spanish patients were
20 treated with clopidogrel [30]. For the same reason all patients from Serbia were excluded from the
21 analysis of prasugrel vs clopidogrel, since no patients in Serbia received prasugrel. To improve
22 balance in distribution of propensity scores in the treatment groups, patients with a propensity score
23 < 0.1 and > 0.9 were excluded [20].

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

1 **Results**

2 **Animal experiments – Infarct size**

3 IPC significantly reduced infarct size compared to controls ($26\pm 12\%$ vs $52\pm 8\%$, $p<0.0001$) (Figure
4 1). RIC also reduced infarct size compared to controls, but not to the same degree as IPC (41 ± 11 vs
5 $52\pm 8\%$, $p<0.05$).

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

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

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

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

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

22

23

24

1 **Clinical study – infarct size**

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

9
10 **Clinical study - outcome**

11 Out of 5115 patients included in the original CONDI-2/ERIC-PPCI study, we included a total of 1754
12 patients in the retrospective main analysis (Figure 2). Of these 395 patients received clopidogrel,
13 1210 patients received ticagrelor and 149 received prasugrel. The number of patients differs between
14 the groups, as patient are included based on the propensity scores. Baseline characteristics of the
15 patients included in the analysis are shown in table 1. All three P2Y₁₂ inhibitors were only prescribed
16 in the UK. Thus, only UK patients were ultimately included in the multinomial logistic regression
17 analysis. Baseline characteristics were generally well balanced. Patients with previous myocardial
18 infarction were slightly more prevalent in the groups treated with ticagrelor (37.6%) and prasugrel
19 (41.6%) compared to clopidogrel (25.6%). Nitrates were used more often in patients treated with
20 clopidogrel (89.4%) and prasugrel (92.6%) compared to ticagrelor (81.3%), which may be due to
21 regional differences in medication strategy.

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

1 95% CI 0.43-1.63) (Table 2) with the time course specified in Figure 3. In analyses of the individual
2 components of the composite primary endpoint ticagrelor reduced the risk to a similar extent, but not
3 all with statistical significance: one-year risk of hospitalization for heart failure (HR 0.57, 95% CI
4 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-
5 1.00) (Table S4). The individual components of the primary endpoint were not affected by prasugrel:
6 one-year risk of hospitalization for heart failure (HR 0.98, 95% CI 0.48-1.99), cardiac death (HR
7 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
8 risk of MACE was not affected by ticagrelor (HR 0.86, 95% CI 0.56-1.32) or prasugrel (HR 0.54,
9 95% CI 0.24-1.23) compared to clopidogrel (Table 2 and Figure 3). Reinfarction, stroke or
10 revascularization did not differ between groups (Table S4).

11 The thirty-day risk of cardiac death or hospitalization for heart failure was reduced in
12 patients receiving ticagrelor (HR 0.64, 95% CI 0.42-0.98), but not prasugrel (HR 0.80, 95% CI 0.39-
13 1.65) compared to clopidogrel (Figure 3 and Table S5). No reduction in MACE was found at 30
14 days.

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

1 **Discussion**

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

8 9 **Effect of P2Y₁₂ inhibitors on infarct size**

10 Beyond the documented beneficial antithrombotic effects on myocardial damage [19,
11 41, 42, 50, 53, 67, 70, 78], experimental studies have shown that second and third generation P2Y₁₂
12 inhibitors may be capable of reducing infarct size in experimental settings, but their cardioprotective
13 capacity appears variable [9, 25, 66, 72, 74, 76]. Translation into a potential clinical effect was already
14 demonstrated for the second generation P2Y₁₂ inhibitor, clopidogrel, but appears to vary as well [24,
15 51].

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

23 Among the orally administered P2Y₁₂ inhibitors, ticagrelor has most convincingly
24 demonstrated infarct size reduction in STEMI patients undergoing rapid revascularization using

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

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

20

21 **Effect of P2Y₁₂ inhibitors on clinical outcome**

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

1 inhibition than with clopidogrel. A statistically significant improvement by prasugrel compared to
2 clopidogrel treatment was not evident from our main endpoint. Consistent with our results a
3 prespecified substudy of the ISAR REACT 5 trial [53] in STEMI-patients demonstrated no significant
4 difference in the primary endpoint (incidence of death, myocardial infarction, or stroke at 1 year after
5 randomization) between prasugrel and ticagrelor [4]. The endpoint in the ISAR REACT 5 study
6 mainly relates to the antithrombotic effect of the P2Y₁₂ inhibitors. We observed a reduction of cardiac
7 death or hospitalization for heart failure by ticagrelor that emerged early compared to clopidogrel but
8 compared to prasugrel most clearly after 180 days of follow-up (Figure 3a and b). The early effect
9 may reflect a superior antithrombotic efficacy of prasugrel and ticagrelor compared to clopidogrel,
10 whereas infarct size reduction by ticagrelor becomes evident with a delay when inappropriate
11 remodeling due to a significant MI size translates into clinical symptoms.

12

13 **Mechanistic considerations**

14 In observational post hoc analyses, the effect of the third generation P2Y₁₂ inhibitors,
15 ticagrelor and prasugrel, versus the second-generation inhibitor, clopidogrel, on microvascular
16 obstruction is equivocal [36, 65]. Ticagrelor does not seem to be superior to prasugrel in reducing
17 microvascular obstruction [62, 64]. Although experimental data suggest that P2Y₁₂-receptor
18 inhibition using cangrelor at the onset of reperfusion can itself reduce MI size [75], it is unclear
19 whether the cardioprotective effect is mediated on the coronary vasculature or the cardiomyocyte [28,
20 36, 37]. Ticagrelor increases circulating levels of adenosine in humans mainly at doses higher than
21 standard [61]. Still, increased serum concentration of adenosine seems to be responsible for
22 ticagrelor-related adverse effects, including dyspnea, ventricular pauses, and bradyarrhythmias.
23 Moreover, experimental as well as human studies suggest that ticagrelor enhances the biological
24 effects of endogenous adenosine [63, 69], implying that adenosine may serve as a mediator of some

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

7 Studies of cardioprotection by P2Y₁₂ inhibitors imply that activated platelets are
8 involved although interference with platelet aggregation itself [18, 48, 73] or improved early coronary
9 reperfusion [24] may not be the main targets. Despite faster P2Y₁₂ inhibition by cangrelor, compared
10 to ticagrelor, this does not necessarily induce an increased salvage of myocardium [60]. Near-
11 obliteration of circulating platelets either with cell poison or an antibody abrogates the
12 cardioprotective effect of P2Y₁₂ antagonists [18]. Furthermore, P2Y₁₂ antagonists have no effect in
13 isolated hearts perfused with platelet-free buffer [18, 73]. Platelets may be a target of P2Y₁₂
14 antagonists for creation of a cardioprotective effect [7]. However, it is unknown whether activated
15 platelets release substances with protective effects on the endothelium and how events between
16 binding of the P2Y₁₂ blocker to its platelet receptor relates to emergence of cardioprotection.

17 Platelet reactivity declines relatively slowly after oral administration of P2Y₁₂ inhibitors
18 and requires several hours before reaching full effect. The profile is most favorable for oral ticagrelor
19 or prasugrel administration as manifestation of the antiplatelet activity within 1-3 hours [2, 8, 10, 26]
20 is less than for clopidogrel for which the effect initiates after 6-11 hours [8]. It remains unknown
21 whether the timing of P2Y₁₂ inhibitors in the CONDI2-PPCI trial was optimal. In the original trial
22 we only had access to data on the type of P2Y₁₂ inhibitor treatment given in relation to the PPCI and
23 not the timing of the administration or the treatment strategy after the index event. Nonetheless,
24 ticagrelor improved the main endpoint indicating that the effect was sufficient.

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Interaction of P2Y₁₂ inhibitor treatment and ischemic conditioning

In preclinical studies, ischemic pre- and postconditioning conditioning interact with the cardioprotective effect of the P2Y₁₂ inhibitor cangrelor, with no additional effect of combination therapy of P2Y₁₂ 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 P2Y₁₂ 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.

1 **Clinical implications**

2 The signal indicating that ticagrelor has the most potent cardioprotective capacity among currently
3 recommended oral P2Y₁₂ inhibitors seems to contribute to the improvement in clinical outcome by
4 modern standard care of patients with STEMI. The achieved improvement in clinical outcome may
5 challenge the ability to document adjunctive cardioprotective treatments beyond optimized standard
6 care in future studies. We have realized this challenge when changing from clopidogrel to ticagrelor
7 in our previous clinical studies of RIC [14, 29]. Careful selection of high-risk patients [11, 12] and
8 multitarget cardioprotective strategies may increase the protective potential [23, 33]. Since
9 intravenous cangrelor may have similar cardioprotective efficacy as ticagrelor, an alternative
10 treatment strategy might be intravenous cangrelor infusion shortly prior to stenting followed by
11 subsequent post-PCI transition to an oral agent [17].

12 **Study limitations**

13 To investigate the cardioprotective capacity of P2Y₁₂ inhibitors we chose an *in vivo* rat model, as the
14 P2Y₁₂ inhibitors necessitate *in vivo* metabolization of the drugs and presence of platelets. Since IPC
15 was induced by an invasive procedure, the experimental design was limited by the prolonged surgical
16 procedure in these groups. Our unpublished pilot trials showed no impact on infarct size from a
17 prolonged surgical procedure in control animals. As IPC and RIC may have different signaling
18 profiles, we cannot exclude an effect of the timing between IPC or RIC and index ischemia. Doses
19 of P2Y₁₂ inhibitors relied on results from other laboratories [25, 44, 66, 72, 74, 76], so we did not
20 conduct dose-response experiments and it cannot be excluded that other doses of P2Y₁₂ inhibitors
21 might increase the cardioprotective effects.

22 The main limitation of the clinical part of the study is that the patients in the original
23 CONDI-2/ERIC-PPCI trial were not randomized by P2Y₁₂ inhibitor prescription. To reduce
24 confounding, our statistical analyses rely on propensity scored analyses. To balance the distribution

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

9

10 **Conclusion**

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

16

1 **Declarations**

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13

14 Conflicts of interest

15 The authors declare no conflicts of interest.

16

17 Ethics approval

18 The ethical approval of the animal experiments of the study was approved by the
19 Danish Veterinary and Food Administration (Authorization number: 2018-15-0201-
20 01475). The clinical part of the study was authorized on ClinicalTrials.gov as
21 NCT02342522.

22

23 Availability of data and material

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

25

1 Code availability

2 Not applicable.

3

4 Author contributions

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

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

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

11

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

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1 **Figure titles and legends**

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

5 **Figure 2 Flowchart.** Flowchart of patient selection and exclusions.

6 **Figure 3 Graphical presentations.** a) the composite endpoint of cardiac death and b) hospitalization
7 for heart failure, and major adverse cardiovascular events, c) and d) display 30-day curves of the same
8 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.