A Comprehensive Review of the Pleiotropic Effects of Ticagrelor

Introduction

Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ receptor inhibitor (i.e., prasugrel, ticagrelor, or clopidogrel) is the cornerstone of treatment for acute coronary syndrome (ACS). The latest European Society of Cardiology guidelines recommended ticagrelor or prasugrel over clopidogrel in ACS patients treated with either invasive or conservative measures (Class IB recommendation) [Collet, Ibanez]. The preference for ticagrelor over clopidogrel is informed by the landmark *Platelet Inhibition and Patient Outcomes* (PLATO) study, which demonstrated superiority of ticagrelor to reduce a composite outcome of death from vascular causes, myocardial infarction (MI), or stroke (driven by significant reductions in both vascular mortality and MI events) without an increased risk for major bleeding events compared to clopidogrel [Wallentin]. Reduction in the primary outcome with ticagrelor versus clopidogrel was observed in ST-elevation myocardial infarction (STEMI) patients treated with percutaneous coronary intervention (PCI), and in non-ST-elevation myocardial infarction (NSTEMI) managed with or without revascularization [Steg, Cannon, Lindholm].

However, subsequent clinical trials and meta-analyses comparing ticagrelor to clopidogrel failed to replicate the positive results seen with the PLATO trial [Gupta, Zhao, Abusnina, Tarantini, Baldetti]. Notably, these subsequent trials and meta-analyses did not account for two crucial observations from the PLATO trial. First, the positive effects of ticagrelor were absent in North American and U.S. patients who received maintenance therapy with high-dose compared to lower-dose aspirin [Mahaffey]. Second, these effects were also attenuated in those off lipid-lowering drugs [Wallentin]. Understanding these interactions may provide the key to uncovering why subsequent trials and meta-analyses comparing ticagrelor to clopidogrel have yielded negative results.

Several explanations for the positive results of PLATO are possible (Figure 1). The predominant hypothesis is that ticagrelor causes faster and greater platelet inhibition. In the acute setting this would translate to better tissue perfusion, and chronically to prevention of subsequent ischemia such as stent thrombosis. However, it is hard to see how these explanations account for the interactions observed in the PLATO trial between ticagrelor, and aspirin and statin therapies. Clinical trials cannot distinguish between acute versus chronic effects of ticagrelor nor temporal interactions with other therapies. Therefore, clinical trials cannot provide much insight into the mechanisms responsible for their outcomes. Preclinical studies can better establish a temporal relationship between treatment and effects, as well as provide evidence for possible mechanisms for the outcomes observed [GD Birnbaum]. This review aims to synthesize preclinical findings on the benefits of ticagrelor with that from the seminal clinical trials and meta-analyses. In doing so, we hope to provide novel insights into the mechanisms of ticagrelor, its benefits over other $P2Y_{12}$ receptor inhibitors, and the failure of clinical trials to reproduce the original results of PLATO.

Effects of a loading dose of ticagrelor when given prior to reperfusion

Patients in clinical trials assessing $P2Y_{12}$ receptor inhibitors are administered a loading dose followed by maintenance treatment, and evaluated for adverse cardiovascular events months to years after initiation of therapy. Thus, they cannot differentiate how the initial loading dose of ticagrelor given prior to reperfusion and chronic maintenance dosing after reperfusion affect ischemic outcomes. In contrast, preclinical trials can isolate and assess the effects of just an acute loading dose or chronic therapy on cardiovascular events. Several animal studies have evaluated the effects of a single loading dose of ticagrelor after induction of MI and prior to reperfusion, in essence mimicking a clinical scenario of acute coronary artery occlusion in the setting of a STEMI. In doing so they offer unique insights into the mechanisms responsible for the benefits seen with ticagrelor loading over other $P2Y_{12}$ therapies.

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Does greater platelet inhibition from acute loading with ticagrelor explain its benefits?

Adenosine diphosphate (ADP) is necessary to activate the glycoprotein IIb/IIIa receptor on platelets, which leads to enhanced platelet degranulation, thromboxane production, and prolonged platelet aggregation. Clopidogrel is a prodrug that requires two-step activation by the liver to its active metabolite before irreversibly antagonizing the ADP-binding site on the $P2Y_{12}$ receptor, leading to attenuation of platelet aggregation. In contrast, ticagrelor directly and reversibly binds to a site on the $P2Y_{12}$ receptor separate from the ADP-binding site [Damman]. Consequently, ticagrelor inhibits platelet aggregation 0.5-2 hours after ingestion compared to clopidogrel which takes 2-6 hours before onset of action [Collet]. Theoretically, faster initial and more potent platelet inhibition with ticagrelor loading can lead to faster and greater attenuation of the progression of atherothrombosis which improves coronary artery perfusion and may lead to acute benefits in the setting of a STEMI.

However, findings from preclinical trials do not support this theory. In a murine model, Ye et al. administered a single dose of intraperitoneal ticagrelor or clopidogrel to rats 5 minutes prior to reperfusion and assessed their effects on infract size 24 hours after reperfusion. Ticagrelor was superior to clopidogrel in reducing infarct size (IS) *despite similar levels of platelet inhibition and bleeding times* measured 2 hours after reperfusion. This translated to improved myocardial function with ticagrelor over clopidogrel 4 weeks later [Ye-1]. Wang et al. similarly found that regardless of similar levels of effects on platelets, ticagrelor treated canines had a significant reduction in rates of coronary re-occlusion and IS minutes to hours after induced infarction compared to clopidogrel [Wang].

Audia et al. showed that a single dose of ticagrelor 10 minutes prior to reperfusion or cangrelor 10 minutes before reperfusion followed by a continuous infusion equally limited IS at 2 hours and 3 days post-reperfusion [Audia]. These results were corroborated by a recent clinical trial, Ubaid et al. which compared the effects on IS of a loading dose of ticagrelor to cangrelor followed by maintenance therapy with ticagrelor in patients presenting with a STEMI. They found that despite greater platelet inhibition by cangrelor compared to ticagrelor at time of balloon inflation during PCI, there was no improvement in coronary reperfusion or IS 13 weeks later [Ubaid]. Unlike the animal studies, this clinical trial cannot separate the acute from chronic effects of ticagrelor on platelet inhibition.

Cangrelor is an intravenous $P2Y_{12}$ receptor inhibitor with a similar mechanism to ticagrelor. It achieves maximal platelet inhibition within 15 minutes of administration, but requires continuous infusion due to its significantly shorter half-life [Damman]. If faster and more potent platelet inhibition were responsible for attenuation of IS, one would expect for IS to be reduced in response to cangrelor compared to ticagrelor treatment. This was not observed, suggesting that greater platelet inhibition does not likely account for reduced IS with ticagrelor in comparison to clopidogrel therapy demonstrated in Ye et al. and Wang et al. Indeed, mitigating delayed microvascular damage, which evolves over several hours after coronary reperfusion may be more important than platelet inhibition at time of PCI [Allencherril].

Pleiotropic effects of ticagrelor in the acute setting

Ischemia-reperfusion injury (IRI), is myocardial damage that occurs as a result of sudden reperfusion of ischemic cardiac tissue due to several mechanisms including oxidative stress, hypercontractility of cardiac muscle due to excess intracellular calcium, and inflammation [Yellon]. Animal studies have shown that reperfusion injury may account for up to half of the final IS after MI, which is recognized as a major determinant of acute and long-term prognosis in patients with acute MI [Asanuma]. Therefore, mitigating

reperfusion injury may be just as important for attenuating myocardial damage and preserving cardiac function as shortening ischemic time.

Effects of ticagrelor, statin, and aspirin therapy on adenosine-metabolism

Adenosine, a widely circulating molecule known for its vasodilative properties, has been shown to mitigate IRI and apoptosis, in addition to improving myocyte regeneration, contractility, and electrical stability [Damman]. The mechanisms by which adenosine mediate these effects are complex. Adenosine activates various receptors on endothelial cells and cardiomyocytes which lead to increases in cyclic adenosine monophosphate (cAMP) and nitric oxide (NO). These molecules induce vasodilation during ischemia which leads to improved metabolic function in both endothelium and coronary smooth muscle. Ischemic preconditioning, the phenomenon of repeated brief episodes of ischemia preceding sustained ischemia, also protects against IRI via adenosine-mediated activation of adenosine triphosphate (ATP) -sensitive potassium channels [Ishida]. Adenosine also attenuates production of free radicals and pro-inflammatory compounds during ischemia and reperfusion. In animal models, these effects of adenosine have been shown to reduce myocardial stunning and improve long-term cardiac function [Kitakaze].

Unique among the $P2Y_{12}$ receptor inhibitors, ticagrelor has direct effects on adenosine metabolism. Ticagrelor binds to equilibrate nucleoside transporter-1 (ENT-1) on platelets and erythrocytes which blocks reuptake of adenosine by these cells [Cattaneo]. In turn, higher levels of adenosine are available to endothelial cells and cardiomyocytes in the local interstitial space at the site of ischemia. Adenosine then mediates local vasodilation and reduction of free radicals and pro-inflammatory compounds that cause IRI [Kitakaze]. Downstream, adenosine receptor activation leads to cyclooxygenase-2 (COX-2) activation and subsequently higher levels of eicosanoids, prostaglandins, and other anti-inflammatory compounds that have been shown to mediate the cardioprotective effects discussed above [Bolli]. These compounds include 15-epi-lipoxin A4, and the pro-survival kinases: Akt, extracellular signal-regular kinase (ERK) 1/2, and endothelial nitric oxide synthase (eNOS) [Nanhwan].

Statin medications also exert effects on adenosine. Statins upregulate 5'-nucleotidase which leads to more adenosine export outside the cells and into the intestinal space. Similar to ticagrelor, higher local concentrations of adenosine induce adenosine-mediated protection against IRI in endothelial cells and cardiomyocytes [Atar, Sanada]. In contrast, high-dose aspirin which inhibits COX-2 downstream of adenosine receptor activation, has been shown to inhibit beneficial late-phase preconditioning and abrogation of myocardial stunning in rabbits [Shinmura].

Preclinical Trials

Several animal studies highlight the importance of ticagrelor's effects on adenosine and its ability to protect against IRI. Previously discussed, Ye et al. comprehensively evaluated the effects of an acute loading dose with ticagrelor versus clopidogrel on IS and myocardial function. When administered to rats intraperitoneally 5 minutes prior to reperfusion, only ticagrelor increased levels of adenosine and the pro-survival kinases, Akt, ERK 1/2, and eNOS. This single dose correlated with reduced IS 24 hours after reperfusion despite similar levels of platelet inhibition 2 hours after reperfusion, and preserved left ventricular diastolic and systolic function 4 weeks later [Ye-1]. In the clinical setting, this study indicates that ticagrelor loading of patients with a STEMI prior to reperfusion likely protects against IRI acutely, while also attenuating adverse cardiac remodeling and preserving cardiac function weeks to months later. As adenosine levels were higher among patients treated with ticagrelor, and adenosine is known to mediate protection against IRI, these outcomes are likely secondary to ticagrelor's effects on adenosine

metabolism. Interestingly, similar effects on IS were seen when chronic ticagrelor therapy was initiated after completion of reperfusion injury; this will be discussed in the next section.

Audia et al. also compared the effects on IS of ticagrelor or cangrelor loading with or without coadministration of VX-765. VX-765 is a potent inhibitor of caspase-1, an enzyme which plays an important role in IRI by mediating the production of pro-inflammatory compounds including IL (interleukin)-1β and IL-18. They demonstrated that loading doses of ticagrelor or VX-765 similarly attenuated IS at both 120 minutes and 3 days after reperfusion, with significant additive effects when administered together. Therefore, ticagrelor likely mediates protection against IRI via inhibition of pro-inflammatory pathways. Additionally, IS was not significantly different between the combinations of ticagrelor and VX-765 or cangrelor and VX-765 [Audia]. Most of the IS limiting effects were seen within the first 120 minutes of treatment which reinforces the conclusion of Allencherril et al. that protection against IRI within the first few hours after coronary reperfusion is vital [Allencherril].

The advantages of ticagrelor over clopidogrel when administered following infarction and prior to reperfusion were also demonstrated in a canine model. In Wang et al., dogs were administered either clopidogrel by IV bolus, ticagrelor by bolus then continuous infusion, or IV saline (control) 25 minutes after induction of MI. A fibrinolytic agent, tissue plasminogen activator (tPa) was co-administered. Despite similar inhibition of platelet aggregation, the ticagrelor group had a significant reduction in rates of coronary re-occlusion, quicker return to baseline coronary blood flow with reduced cyclic flow variation, and reduced IS when compared to clopidogrel at 120 minutes of reperfusion. These effects were seen despite all animals receiving aspirin 325 mg orally on the day of the procedure. [Wang]. A prior canine study showed increased reactive hyperemia, a transient increase in coronary blood flow that occurs following ischemia, when adenosine half-life was extended *in vivo.* [Björkman]. Thus, Wang et al. concluded that the improvement in coronary blood flow was possibly due to ticagrelor inducing adenosine-mediated vasodilation [Wang]. The results of this trial suggest that ticagrelor and not clopidogrel augments fibrinolytic therapy when used for STEMI treatment by improving coronary blood flow and preventing reocclusion via adenosine-mediated vasodilation.

Effects of Chronic Treatment with Ticagrelor

Preclinical trials can also help delineate the effects of ticagrelor when initiated after completion of IRI, simulating treatment of STEMI patients with maintenance $P2Y_{12}$ therapy. The benefits conferred to patients by ticagrelor fall into two major camps: 1) Prevention of future cardiac events, 2) Protection against IRI if infarction occurs while on chronic ticagrelor therapy.

Prevention of future cardiac events

The benefits of chronic ticagrelor therapy over other $P2Y_{12}$ receptor inhibitors may be attributed to its ability to prevent future cardiac events after an MI. The mechanisms by which this may occur include greater platelet inhibition in the long-term leading to less recurrent ischemia, attenuation of inflammation mitigating adverse cardiac remodeling, and/or prevention of atherosclerosis.

Platelet Inhibition with Maintenance Ticagrelor Treatment

We previously discussed that despite its more direct mechanism, greater initial and more rapid platelet inhibition with an acute loading dose of ticagrelor is likely not responsible for its IS attenuating effects. In the chronic setting, it is also possible that greater long-term platelet inhibition could prevent the formation of atherothromboses and subsequent ischemia. A subgroup analysis of 69 PLATO subjects found that after 28 days of treatment, markers of platelet reactivity were higher among those who

received clopidogrel compared to ticagrelor. [Storey]. However, this was a non-prespecified post-hoc analysis that was unable to comment on whether this observation correlated with improved outcomes.

Preclinical trials comprehensively evaluated whether more potent platelet inhibition with chronic ticagrelor versus clopidogrel treatment leads to better protection against future cardiac events. In Nanhwan et al., after 7 days of pre-treatment with ticagrelor and clopidogrel, levels of platelet aggregation were not significantly different in the blood of rats treated with ticagrelor versus clopidogrel. Despite this, only ticagrelor reduced IS after 24 hours of reperfusion [Nanhwan]. Birnbaum et al. yielded similar results comparing ticagrelor to prasugrel after 3 days of dosing [Birnbaum Y-1]. Another murine study demonstrated improved left ventricular function measured by echocardiography at days 14 and 28 with ticagrelor compared to prasugrel therapy started 7 days after ischemia and reperfusion [Birnbaum Y-2]. An additional preclinical trial observed attenuation of atherosclerosis progression with ticagrelor or rosuvastatin compared to clopidogrel after 14 weeks of treatment. [Ye-2]. All of these results were seen in the setting of similar levels of platelet inhibition with ticagrelor compared to other $P2Y_{12}$ receptor inhibitors. Thus, just as with acute ticagrelor loading, greater and more potent platelet inhibition in the chronic setting does not appear to explain the benefits of reduced IS, attenuation of adverse remodeling, mitigation of atherosclerotic progression, or protection from IRI when infarction occurs on chronic ticagrelor treatment. These trials are discussed in depth in the following sections.

Attenuation of Inflammation and adverse cardiac remodeling

Adenosine also protects against adverse cardiac remodeling. An increased neurohormonal response after infarction induces a release of catecholamines and growth factors which lead to fibrosis, beta-adrenoceptor mediated myocardial hypercontractility, and myocyte hypertrophy [Sutton]. Adenosine leads to a reduction in the release of catecholamines and calcium overload, and also augments coronary blood flow and inhibition of platelet and leukocyte activation via activation of adenosine receptors. Adenosine has also been shown to inhibit renin release and tumor necrosis factor (TNF)-α production in experimental models, processes that contribute to adverse cardiac remodeling [Kitakaze].

The previously discussed Ye et al. study assessed not only the effects of an acute loading dose of ticagrelor on IS in rats, but also the outcome of myocardial function from chronic ticagrelor treatment initiated after completion of IRI. They demonstrated that 4 weeks of dosing started 24 hours after reperfusion normalized left ventricular internal diameter in diastole and systole, and preserved left ventricular ejection fraction and fractional shortening similar to a loading dose of ticagrelor. In the group of rats that were treated with both a loading dose and chronic treatment after reperfusion, there was additive effect to improve these echocardiographic parameters. These effects were not seen with clopidogrel. The study also showed increased levels of adenosine and pro-survival kinases, as well as a reduction in fibrosis and decreased inflammatory cytokines including TNF-α, IL-1β, and IL-18 in ticagrelor compared to clopidogrel-treated rats. Similar preservation of cardiac function with acute and chronic dosing suggests that the benefits of maintenance ticagrelor therapy are independent of protection from IRI. Rather, chronic dosing attenuates inflammation and prevents adverse cardiac remodeling as evidenced by decreased inflammatory cytokines and markers of fibrosis correlating with preserved myocardial function. These pathways are likely adenosine-mediated [Ye-1].

Birnbaum et al. similarly demonstrated that ticagrelor prevents adverse cardiac remodeling when initiated even longer (7 days) after completion of IRI [Birnbaum Y-2]. In this study, rats were administered daily doses of oral ticagrelor, aspirin, both ticagrelor and aspirin, or prasugrel 7 days after infarction and reperfusion. At 14- and 28-days after reperfusion, both ticagrelor and aspirin attenuated the decrease in systolic function and remodeling as evaluated by echocardiography. Ticagrelor-treated rats also had smaller increases in serum markers of remodeling including atrial and brain natriuretic peptides, collagen-I and collagen-III, and increased mRNA levels and cell expression of various markers of proliferation and

progenitor stem cells in the infarcted tissue. Notably, these effects were not seen with prasugrel or when aspirin and ticagrelor were administered together, suggesting that aspirin may block the effects of ticagrelor to limit adverse cardiac remodeling and cardiomyocyte regeneration. Furthermore, both ticagrelor and aspirin independently increased levels of the eicosanoid, 15-epi-lipoxin-A_4, an effect that was blunted when ticagrelor and aspirin were concomitantly administered. This suggests that the diminution of IS and inflammatory and fibrotic markers by ticagrelor are at least partially mediated via 15-epi-lipoxin-A_4 which is activated downstream from adenosine receptor and COX-2 activation; this may be inhibited by concomitant chronic aspirin therapy.

Atherosclerosis

Recently, the pathogenesis of atherosclerosis is realized to be mediated by complex interactions between endothelial cells and the immune system [Libby]. Essentially, cell adhesion molecules recruit leukocytes to the inflamed vascular tissue which then release various pro-inflammatory cytokines which aid with deposition of cholesterol in the arterial walls, and worsens atherosclerosis progression in a "vicious cycle" [Manduteanu]. The following studies show that ticagrelor, which demonstrates adenosine-mediated attenuation of pro-inflammatory cytokines, may mitigate the progression of atherosclerosis.

In a murine study, Preusch et al. investigated the effects of ticagrelor supplementation for 25 weeks in advanced atherosclerotic apolipoprotein-E-deficient (Apo-E-/-) mice with an atherosclerotic lesion in the aortic sinus. The mice treated with ticagrelor had a significant reduction in the relative area of the necrotic core and increase in fibrous cap thickness. In an *in vitro* analysis, mice that received ticagrelor also had a significant reduction in the prevalence of apoptotic macrophages and their uptake of oxidized lipoprotein lipase (oxLDL) [Preusch]. Another study showed this reduction of oxLDL was dose-dependent, and that ticagrelor also decreased expression of proprotein convertase subtilisin/kexin type (PCSK9), a powerful regulator of low-density lipoprotein (LDL) receptor degradation [Xia].

Another mouse model evaluated the effects of ticagrelor versus clopidogrel on atherosclerotic development. In this study, hypercholesterolemic mice were fed a high-fat diet and given clopidogrel or ticagrelor for 16 weeks. Despite doses resulting in equal levels of platelet inhibition, the ticagrelor group had significantly less atherosclerosis, as evidenced by less macrophage infiltration of the atherosclerotic intima, and lower serum levels of pro-atherosclerotic markers. An additional marker of interest, paraoxonase-1, which inhibits proinflammatory cytokines that lead to atherosclerosis, had higher serum activity and tissue levels in the ticagrelor compared to clopidogrel group [Halim]. This study highlights not only ticagrelor's ability to mitigate atherogenesis, but also its increased efficacy over clopidogrel via non-platelet mediated lowering of pro-inflammatory cytokines.

Ye et al. then assessed whether aspirin can also block the anti-atherogenic effects of ticagrelor. Diabetic mice received rosuvastatin, aspirin, ticagrelor, clopidogrel, or their combination for 14 weeks after which 15-epi-lipoxin-A_4, IL-1β, IL-6, TNF-α, aortic wall cholesterol content, and atherosclerotic plaque area were assessed. Aspirin, ticagrelor, and rosuvastatin each independently increased 15-epi-lipoxin-A_4 and decreased IL-1β, IL-6, TNF-α, as well as atherosclerotic plaque area. The combination of rosuvastatin and ticagrelor augmented the increase of anti-inflammatory and decrease of pro-inflammatory cytokines and atherosclerotic plaque area. Again, aspirin was demonstrated to interfere with the attenuation of inflammatory cytokine levels by both ticagrelor and rosuvastatin with the exception of IL-1β and IL-6 [Ye-2]. In essence, the combination of ticagrelor and rosuvastatin demonstrated a synergistic effect of mitigating inflammatory cytokine levels and progression of atherosclerosis that were not exhibited with clopidogrel use. These effects were lost in the presence of aspirin. Thus, the pleiotropic effect of ticagrelor to reduce inflammation and atherosclerotic progression was shown to likely be at least partially due to adenosine-mediated increases in 15-epi-lipoxin-A_4.

Protection against IRI from recurrent infarction

A robust set of preclinical trials assessed whether the acute effects of ticagrelor to protect against IRI were evident when ischemia occurs while on chronic ticagrelor treatment. They also assessed the effects of aspirin and statin medications on the ability of maintenance ticagrelor therapy to reduce IS and preserve myocardial function. Caffeine is perhaps the most widely used adenosine receptor antagonist [Ribeiro]. Studies also evaluated whether caffeine affects the pleiotropic benefits of ticagrelor and statin therapy.

In Nanhwan et al., rats were pre-treated with ticagrelor or clopidogrel for 7 days, then underwent 30-minute coronary ligation flowed by 24-hour reperfusion. Myocardial IS was significantly reduced in rats treated with ticagrelor but not with clopidogrel, with similar levels of platelet inhibition. This was showed to be due to higher levels of adenosine in ticagrelor-treated myocardium, leading to adenosine receptor activation and downstream up-regulation of eNOS, cytosolic phospholipase (cPLA2), COX-2, and 15-epi-lipoxin-A_4. Notably, the myocardial IS limiting effect of ticagrelor was attenuated when an adenosine receptor antagonist, COX-2 inhibitor, or high-dose aspirin were given one hour before ischemia-reperfusion in a dose-dependent manner. However, no attenuation was seen when a COX-1 inhibitor or low-dose aspirin were given [Nanhwan]. This study indicates that high-dose aspirin as is given as standard of care in the setting of ACS likely blocks the effects of ticagrelor to protect against IRI if ischemia occurs while on maintenance therapy. In addition, aspirin likely does this by inhibiting adenosine-mediated COX-2 upregulation and downstream activation of 15-epi-lipoxin-A_4 and other anti-inflammatory compounds known to contribute to protection from IRI.

In the previously referenced Birnbaum et al., rats received either ticagrelor, prasugrel, rosuvastatin, ticagrelor plus rosuvastatin, prasugrel plus rosuvastatin, or water as a control for 3 days, after which they were subjected to 30-min coronary artery occlusion and 24 hours of reperfusion. The combination of rosuvastatin and ticagrelor, but not prasugrel, demonstrated an additive effect of increasing adenosine levels and reducing IS more than either rosuvastatin or ticagrelor alone. These outcomes were not observed in the group given ticagrelor and rosuvastatin in addition to an adenosine receptor antagonist one hour before coronary artery occlusion. Furthermore, only ticagrelor and rosuvastatin increased expression of COX-2, 5-epi-lipoxin A_4, and the pro-survival kinases, Akt, ERK 1/2 , and eNOS which contribute to protection from reperfusion injury [Birnbaum Y-1]. Both ticagrelor and rosuvastatin (not prasugrel) also significantly attenuated the increase of caspase-1 following ischemia-reperfusion corroborating the findings of Audia et al. [Audia]. An earlier study showed that when aspirin was administered to rats pretreated with atorvastatin 15 minutes prior to reperfusion, IS reduction by atorvastatin was blunted [Birnbaum Y-3]. These studies suggest that aspirin loading prior to PCI may block the adenosine-mediated effects of both maintenance ticagrelor and statin therapy to protect against IRI.

Another murine study evaluated whether, like aspirin, caffeine blocks the pleiotropic effects of ticagrelor and statin medications. Rats were administered atorvastatin or water as a control for 3 days, along with sugar water, caffeinated coffee with sugar, or decaffeinated coffee with sugar. On day 4, 30-minute coronary artery occlusion was induced followed by 4-hour reperfusion. IS was significantly reduced in the atorvastatin groups that received sugar water and decaffeinated coffee, but not in the caffeinated coffee group [Ye-3]. This was shown to be due to blocking Akt phosphorylation by atorvastatin leading to less eNOS activation and attenuation of the myocardial protective effects of statins against IRI. The results of this study suggest that the chronic effects of statins to defend against IRI as demonstrated in Birnbaum et al., can be attenuated by adenosine antagonism by caffeine. This likely applies to ticagrelor therapy as both mechanisms are adenosine-mediated. Caffeine inhibition of these beneficial pleiotropic effects of ticagrelor and statin therapy may be compounded by chronic low-dose aspirin as it still causes some COX-2 inhibition, but this question remains unanswered.

Liu et al. examined whether acute and chronic treatment with ticagrelor could prevent IRI from recurrent ischemia. In this study, rats were administered either ticagrelor immediately after LAD ligation followed by daily maintenance dosing or saline. A subgroup of these rats were pre-treated with dextran sodium sulfate (DSS, a known NF-kB agonist) for 7 days prior to MI induction. The LAD was then re-ligated at 24 hours, 3, and 7 days after initial ligation and inflammatory markers including NF-kB, galectin-3, IL-6, and TNF- α were measured in cardiomyocytes. Ticagrelor significantly reduced IS and downregulated NF-kB, galectin-3, IL-6, and TNF- α at each time interval. Pretreatment with DDS attenuated these effects suggesting that these effects of ticagrelor to protect against IRI due to recurrent ischemia may be partly mediated by inhibiting the activation of NF-kB in the ischemic myocardium [Liu]. However, adenosine can inhibit NF-kB [Li]. Thus, whether reduction of NF-kB represents a separate mechanism by which ticagrelor improves myocardial function in the setting of IRI, or it is further evidence of its adenosine-mediated effects has yet to be determined.

Hjortbak et al. assessed whether a single dose of ticagrelor given prior to infarction could attenuate the detrimental effects of IRI on the rat heart. Either ticagrelor 2 hours, prasugrel 2 hours, or clopidogrel 4 hours prior to infarction was administered to ensure similar platelet inhibition. A loading dose of ticagrelor demonstrated reduced IS compared to control after 2 hours of reperfusion. This was not seen with prasugrel or clopidogrel. Additionally, there was no additive effect with ischemic preconditioning indicating that either ticagrelor may already partially work via this mechanism, or that ischemic preconditioning is less clinically significant when infarction occurs on chronic ticagrelor therapy [Hjortbak]. Thus, even a single dose of ticagrelor likely protects against IRI prior to an ischemic event, which is evidence of its potent cardioprotective properties.

Similar studies were performed in porcine models of IRI. Vilahur et al. also demonstrated that a single dose of ticagrelor given 2 hours prior to induced infarction in pigs reduced IS as evidence by cardiac MRI (CMR) compared to clopidogrel. These was also less edema and necrosis seen on CMR with ticagrelor versus clopidogrel. Concomitant administration of an adenosine receptor antagonist mitigated these effects seen with ticagrelor loading. [Vilahur-1]. In a second study, IS of pig hearts was measured on CMR after they were administered a loading dose of ticagrelor or clopidogrel prior to MI induction – followed by 42 days of chronic treatment. Only ticagrelor showed reduced IS, scar formation, edema, and attenuated reduction in left ventricular ejection faction 3 days after reperfusion. These effects persisted with repeat imaging on day 42. Similar to the findings of Ye et al. and Nanhwan et al., ticagrelor also led to higher adenosine levels [Vilahur-2] These trials further add to the cannon of animal studies in mammals indicating that ticagrelor protects against IRI with a single loading dose, and adverse remodeling with both acute and chronic treatment via adenosine-mediated mechanisms.

Adenosine and its downstream effects on COX-2 and 15-epi-lipoxin-A_4 have been shown to mediate pleiotropic effects of chronic ticagrelor therapy including: attenuation of inflammation and adverse cardiac remodeling, prevention of atherosclerosis, and protection from IRI. Birnbaum et al., Nanhwan et al., and Vilahur et al. demonstrated that both aspirin and adenosine receptor antagonism interfere with these beneficial effects. Thus, high dose aspirin delivered as standard of care in the event of ACS likely interferes with these beneficial pleiotropic effects. This interaction can be further elucidated by comparing the results of clinical and preclinical trials.

Analyzing clinical trial outcomes through the lens of the findings from animal studies

ATLANTIC

The 2014 *Administration of Ticagrelor in the Cath Lab or in the Ambulance for New ST Elevation Myocardial Infarction to Open the Coronary Artery* (ATLANTIC) trial evaluated whether early administration of ticagrelor en route to the hospital compared to in the catheterization laboratory would have an effect on

ischemic outcomes in the acute setting of a STEMI. In this multicenter randomized double-blind trial of 1,862 patients with ongoing STEMI of less than 6 hours duration, patients were randomly assigned to receive a 180 mg loading dose of ticagrelor either en route in the ambulance or in the catheterization laboratory prior to primary PCI, in addition to aspirin loading and standard of care. Pre-hospital administration of ticagrelor did not improve pre-PCI coronary reperfusion of the culprit artery nor ST-segment elevation resolution on electrocardiogram (ECG) 1-hour post-PCI. [Montalescot].

The original hypothesis of the study investigators was that earlier ticagrelor administration would cause faster and stronger platelet inhibition, and lead to quicker resolution of acute coronary occlusion. However, as has been demonstrated by preclinical trials, ticagrelor's acute cardioprotective effects are mostly due to protection from IRI rather than more potent platelet inhibition. The authors explained the lack of efficacy in the primary outcomes by a clinically non-significant time-to-PCI difference in both groups; the median time from randomization to angiography was 48 minutes, and the median time difference between the prehospital and in hospital group was only 31 minutes. Given the effects of increased coronary blood flow and reduced IS demonstrated in several animal studies, it would be expected that these findings would correlate with quicker resolution of ST-segment elevation or improved reperfusion. However, it is possible that 31 minutes was not enough time to make a clinical difference in protection from IRI. Another plausible explanation is that high-dose chewable aspirin that was administered to all patients as standard of care for ACS blocked the ability of ticagrelor to induce protective coronary vasodilation, and attenuate IRI via down-regulation of COX-2 and subsequent production of protective eicosanoids and prostaglandins.

Clinical Trials Evaluating IS

Following the lead of the previously discussed animal studies, recent clinical trials analyzed the effects of ticagrelor and other $P2Y_{12}$ receptor inhibitors on IS. Ubaid et al., compared the effects on IS of a loading dose of ticagrelor to cangrelor followed by maintenance therapy with ticagrelor in patients presenting with a STEMI. They found that despite greater platelet inhibition by cangrelor compared to ticagrelor at time of balloon inflation during PCI, there was no improvement in coronary reperfusion or IS 13 weeks later [Ubaid]. If greater platelet inhibition were responsible for reduced IS, smaller IS would be expected in patients treated with cangrelor. However, no difference was observed. IRI contributes to half of final IS. [Asanuma]. Ticagrelor has been demonstrated to protect against IRI acutely, and adverse cardiac remodeling with maintenance therapy after MI, independent of one another. Therefore, it may be expected that patients who received ticagrelor would have had reduced IS. However, all patients received both aspirin loading prior to PCI and daily maintenance therapy which have been shown to inhibit ticagrelor's ability to protect against IRI, and attenuation of adverse cardiac remodeling respectively. [Nanhwan, Birnbaum Y-2]. Thus, it is possible the lack of difference in IS was a result of aspirin blocking these pleiotropic effects of ticagrelor in both the acute and chronic setting.

Khan et al. conducted a randomized study on the effects of clopidogrel versus ticagrelor or prasugrel loading before primary PCI. IS was smaller and myocardial salvage greater at 3 days in the ticagrelor or prasugrel compared to the clopidogrel group [Khan]. In a retrospective analysis of the *DANish Study of Optimal Acute Treatment of Patients With ST-elevation Myocardial Infarction* (DANAMI)-3 trial, Sabbah et al. found similar results at 3 month follow up. However, these trials did not separately analyze patients given prasugrel and ticagrelor, and so conclusions about the effects of each individual therapy cannot be discerned [Sabbah]. Furthermore, Ubaid et al. and Sabbah et al. evaluated IS at 3 months in patients who received both acute and chronic ticagrelor therapy. Thus, acute protection from IRI and chronic effects of ticagrelor to prevent cardiac remodeling as demonstrated in the animal studies cannot be determined.

A Comprehensive Review of the Pleiotropic Effects of Ticagrelor

PLATO

The PLATO trial randomized 18,624 patients with ACS – with or without STEMI – treated with invasive or ischemia-guided management to either ticagrelor or clopidogrel. The study drug was administered within a mean of 11.3 hours from start of symptoms and 5 hours within time of randomization. All patients received at least 75-100 mg aspirin daily unless they could not tolerate it, and a 325 mg loading dose if they were not previously taking prior to randomization. Treatment with ticagrelor compared to clopidogrel resulted in significant reduction in the composite primary endpoint of death from vascular cause, MI, or stroke after 12 months (9.8% vs. 11.7%; Hazard Ratio [HR] 0.84, 95% CI 0.77 – 0.92; $p < 0.001$) while no significant difference was observed in the rates of major bleeding found between the two groups [Wallentin]. Reduction in the primary outcome with ticagrelor compared to clopidogrel was observed in STEMI treated with PCI, and NSTEMI patients managed with or without revascularization [Steg, Cannon, Lindholm]. Ticagrelor's benefits became apparent within the first 30 days of therapy and persisted at 12-month follow-up [Wallentin].

The benefits of ticagrelor demonstrated in the PLATO trial were not homogenously distributed. Subgroup analysis revealed that ticagrelor was associated with more cardiovascular events in the North American (HR 1.25) and U.S. populations (HR 1.27) compared to the rest of the world (0.84). While the possibility of chance occurrence could not be definitively ruled out via statistical analysis, two independent statistical techniques identified maintenance aspirin dose as a possible factor influencing this regional difference. [Mahaffey]. Defined as the median dose of aspirin a patient received from day 2 of hospitalization until cessation of treatment drug, high-dose aspirin (≥300 mg daily) was associated with more adverse cardiovascular outcomes than low-dose aspirin (≤ 100 mg daily) in patients who received ticagrelor. Furthermore, the lowest event rates among those receiving ticagrelor were with the low-dose and greatest in the high-dose aspirin group. These patterns were absent in those who received clopidogrel.

Aside from the statistical results, the authors were unconvinced that high-dose aspirin could be responsible for higher cardiac event rates in ticagrelor-treated patients on high-dose aspirin because of a lack of explanation for the possible interaction. The authors proposed that aspirin at daily doses of >80mg may have attenuated ticagrelor's antiplatelet effects via inhibition of endothelial release of prostaglandins, in a dose-dependent fashion [Mahaffey]. However, preclinical studies demonstrate that quicker and greater platelet inhibition is likely not responsible for ticagrelor's cardioprotective effects. Rather, a robust set of animal studies indicate that via its effects on local adenosine metabolism, ticagrelor protects from IRI in the acute setting, as well as prevention of further cardiovascular evens via attenuation of adverse cardiac remodeling and atherosclerosis. Nanhwan et al. demonstrated that co-administration of high-dose aspirin or COX-2 inhibition block these cardioprotective benefits, while low-dose aspirin did not exhibit the same inhibitory effects [Nanhwan]. Birnbaum et al. and Ye et al. also showed that maintenance aspirin administered with ticagrelor blocks the effects of ticagrelor to prevent adverse cardiac remodeling and atherosclerotic progression respectively [Birnbaum Y-2, Ye-2]. Thus, it is more likely that the geographical difference explored by Mahaffey et al. is due to the interaction of ticagrelor and aspirin, rather than chance alone. Indeed, "The potential adverse effect of aspirin in attenuating protection has not yet been considered seriously in this regard." [Ye R].

Subgroup analysis of PLATO participants on and off lipid-lowering drugs was also performed. The PLATO authors observed that patients who were administered ticagrelor exhibited a significant reduction in mortality while on concomitant therapy with lipid-lowering drugs versus those who were not taking lipid-lowering therapies compared to clopidogrel [Wallentin]. The study did not specify which lipid-lowering agents were used, though presumably these were statins given that >90% of patients on lipid-lowering

drugs at the time of the trial were taking statin medications. [Carroll]. As demonstrated by Ye et al. and Birnbaum et al., via increasing extracellular adenosine levels, statin medications augment ticagrelor's ability to protect against atherosclerosis and IRI. Thus, patients on both ticagrelor and statin medications likely had the added benefit of protection from complications of IRI and future cardiovascular events reflected by a lower event rate compared to patients not taking statin medications. If both of these observations hold true, the most dramatic difference in cardiovascular outcomes would be expected to be between PLATO participants who received low maintenance doses of aspirin on statin therapy compared to patients who received high maintenance doses of aspirin, but no statin. However, no subgroup analysis of high-dose versus low-dose aspirin was done in patients who received statin therapy.

Meta-analyses and Future Direction

Despite, the impressive positive results of the PLATO trial, subsequent clinical trials and meta-analyses have failed to reproduce the benefits seen with ticagrelor. Several meta-analyses concluded that treatment with ticagrelor or prasugrel have marginal effects on major adverse cardiac events and strokes, do not translate into improved overall mortality and bleeding, and may even lead to increased bleeding risk in the elderly population compared with clopidogrel in patients presenting with ACS. [Gupta, Zhao, Abusnina, Tarantini]. One meta-analysis further found that patients who received prasugrel had decreased rates of cardiovascular events compared to ticagrelor [Baldetti]. However, none of these trials commented on or analyzed concomitant use of aspirin or statin medications. Thus, the conclusion of these meta-analyses should not be considered definitive, and can be more accurately characterized as the following: ticagrelor appears to have no benefit compared to other $P2Y_{12}$ receptor inhibitors in the setting ACS when standard of care including aspirin is employed.

The GLOBAL LEADERS trial found that ticagrelor monotherapy for 23 months after 1 month of DAPT is noninferior (but not superior) to 12 months of standard DAPT followed by 12 months of aspirin alone for the prevention of all-cause mortality or recurrent MI after PCI [Vranckx]. If aspirin blocks the pleiotropic effects of ticagrelor to protect against future cardiac events, it may be expected that ticagrelor monotherapy would be superior to DAPT. However, aspirin maintenance therapy was low (75-100 mg daily) which Nanhawan et al. showed is less likely to prevent ticagrelor-mediated protection from future cardiac events compared to high-dose aspirin [Nanhwan]. As demonstrated by Ye et al., concomitant caffeine use also inhibits adenosine-mediated effects of ticagrelor to protect against IRI [Ye-3]. This may extend to other chronic adenosine-mediated effects of ticagrelor, i.e. attenuation of adverse cardiac remolding and atherosclerosis development. Caffeine was not limited in these trials. Theoretically, the combination of low-dose aspirin and daily caffeine may have inhibited these beneficial pleiotropic effects, leading to no detectable difference between ticagrelor monotherapy and aspirin.

In animal models, aspirin has also been shown to limit the IS reducing effects of opiates and ischemic postconditioning [Ye R]. Moving forward, large animal models should evaluate separately the effects of aspirin loading before reperfusion and chronic aspirin therapy at high versus low doses on the effects of ticagrelor to attenuate IS and adverse cardiac remodeling. They should also assess the effects of background therapy with aspirin, statins, caffeine, opiates, and ischemic postconditioning on ticagrelor's pleiotropic effects. If these studies confirm the findings of the preclinical trials discussed in this review, it may be time for clinical trials to compare patients who present with ACS receiving ticagrelor with and without aspirin.

Conclusion

When administered prior to reperfusion, animal studies demonstrate that ticagrelor likely protects against IRI. Preclinical trials also show that chronic treatment with ticagrelor protects against adverse cardiac remodeling, development of atherosclerosis, and protection against IRI from recurrent ischemia. These effects are likely mediated by ticagrelor's ability to increase local interstitial adenosine levels which activate downstream anti-inflammatory prostaglandins and eicosanoids. High-dose aspirin and adenosine-antagonism have been demonstrated to block these effects of ticagrelor, as well as statin's ability to augment them. Attenuation of ticagrelor's adenosine-mediated pleiotropic effects by aspirin likely explains the differential of outcomes among PLATO participants who received high versus low-dose aspirin, and statin versus no statin therapy. Subsequent trials and meta-analyses have not accounted for these interactions. We are in need of more clinical and preclinical trials comparing cardiovascular outcomes in patients who present with ACS treated with ticagrelor versus other $P2Y_{12}$ receptor inhibitors that are mindful of the unique pleiotropic advantages afforded by ticagrelor, and possible interactions that pose a threat to harnessing these benefits.

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