Journal of the American College of Cardiology
© 2014 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 63, No. 23, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.03.031

Adenosine-Mediated Effects of Ticagrelor

Evidence and Potential Clinical Relevance

Marco Cattaneo, MD,* Rainer Schulz, MD, PhD,† Sven Nylander, PhD‡

Milan, Italy; Giessen, Germany; and Mölndal, Sweden

This review constitutes a critical evaluation of recent publications that have described an additional mode of action of the $P2Y_{12}$ receptor antagonist ticagrelor. The effect is mediated by inhibition of the adenosine transporter ENT1 (type 1 equilibrative nucleoside transporter), which provides protection for adenosine from intracellular metabolism, thus increasing its concentration and biological activity, particularly at sites of ischemia and tissue injury where it is formed. Understanding the mode of action of ticagrelor is of particular interest given that its clinical profile, both in terms of efficacy and adverse events, differs from that of thienopyridine $P2Y_{12}$ antagonists. (J Am Coll Cardiol 2014;63:2503–9) © 2014 by the American College of Cardiology Foundation

Ticagrelor is a direct-acting, reversibly binding $P2Y_{12}$ antagonist that provides rapid onset of antiplatelet effects after oral administration. $P2Y_{12}$, 1 of the 2 purinergic receptors for adenosine diphosphate (ADP) expressed by platelets, is essential for normal ADP-induced platelet aggregation. $P2Y_{12}$ signaling amplifies platelet responses to agonists that cause ADP release from delta granules, stabilizes platelet aggregates, and opposes the antiplatelet effects of natural platelet inhibitors such as prostacyclin that induce production of the inhibitory cyclic adenosine monophosphate (cAMP) by activating adenylyl cyclase (1).

The essential role of $P2Y_{12}$ in hemostasis and thrombosis is demonstrated by observations that patients with inherited $P2Y_{12}$ defects have a bleeding diathesis and that the administration of antagonists reduces the incidence of major adverse cardiovascular events in patients at risk (1). The most widely used $P2Y_{12}$ antagonist is the thienopyridine prodrug clopidogrel, which, through hepatic conversion to its active metabolite, irreversibly inhibits $P2Y_{12}$. Compared with clopidogrel, ticagrelor provides higher and much less variable $P2Y_{12}$ inhibition. This is also true for the third-generation thienopyridine prasugrel (1), albeit ticagrelor has been shown to provide slightly greater platelet inhibition (2). In the PLATO (Platelet Inhibition and Patient Outcomes) study, ticagrelor was superior to clopidogrel in preventing cardiovascular death, myocardial infarction, or stroke (9.8% vs. 11.7%, a 16% reduction) in patients with acute coronary syndrome (ACS); two-thirds of these patients had undergone percutaneous coronary intervention (PCI) (3). Prasugrel was also superior to clopidogrel in preventing the same composite endpoint in patients with ACS who underwent PCI in the TRITON (Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel) trial (4). Although the 2 trials had quite different designs, they showed that more rapid, higher, and more consistent $P2Y_{12}$ inhibition than afforded by clopidogrel was associated with better clinical outcome. PLATO and TRITON did display some differences. In PLATO, ticagrelor slightly but significantly reduced the incidence of cardiovascular (4.0% vs. 5.1%) and total (4.5% vs. 5.9%) mortality compared with clopidogrel, whereas no significant reduction in cardiovascular (2.1% vs. 2.4%) or total (3.0% vs. 3.2%) mortality was observed with prasugrel versus clopidogrel in TRITON (3,4). In addition, a greater incidence of dyspnea and ventricular pauses was observed with ticagrelor (3). Although the accuracy of the PLATO mortality data has been questioned by some authors (5), their allegations have been rebutted by the PLATO investigators (6). Thus, although alternative interpretations exist (7), the PLATO data on mortality suggest that ticagrelor may have unique and clinically relevant effects, as also demonstrated by the observed increased incidence of dyspnea and ventricular pauses (3). These effects of ticagrelor may be accounted for by its reversible binding to $P2Y_{12}$, its systemic presence at pharmacologically active concentrations over 24 h of the day (8), and/or by additional $P2Y_{12}$-independent effects (9).

Here, we will review the experimental and clinical evidence that ticagrelor increases the half-life and plasma concentration of adenosine, and critically evaluate whether

From the *Unità di Medicina 3, Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy; †Institute of Physiology, Justus-Liebig University Giessen, Giessen, Germany; and ‡AstraZeneca Research and Development, Mölndal, Sweden. Dr. Cattaneo has received research grants from AstraZeneca, Eli Lilly, Daiichi Sankyo, and Evolva; and has received honoraria for lectures or for service on advisory boards from AstraZeneca, Eli Lilly, Daiichi Sankyo, Evolva, Sanofi-Aventis, and Merck Sharp & Dohme. Dr. Schulz has received research grants from Zealand Pharma and honoraria for lectures and membership on advisory boards from AstraZeneca, Recordati, Sanofi-Aventis, and Servier. Dr. Nylander is an employee of AstraZeneca.

Manuscript received February 4, 2014; revised manuscript received March 21, 2014, accepted March 24, 2014.

JACC Vol. 63, No. 23, 2014
June 17, 2014:2503–9

Abbreviations and Acronyms

ACS = acute coronary syndrome(s)

ADP = adenosine diphosphate

cAMP = cyclic adenosine monophosphate

CNT = concentrative nucleoside transporter

ENT1 = equilibrative nucleoside transporter

PCI = percutaneous coronary intervention

this additional effect of ticagrelor may explain the drug's clinical profile.

Ticagrelor Inhibits Cellular Uptake of Adenosine

Adenosine is a purine nucleoside produced primarily through the metabolism of ADP or adenosine triphosphate by the nucleotidases CD39 and CD73; its plasma levels increase after cellular stresses such as injury, ischemia/reperfusion, or inflammation (10). Adenosine is rapidly taken up by cells through sodium-independent equilibrative nucleoside transporters (ENT 1/2) and sodium-dependent concentrative nucleoside transporters (CNT 2/3) (11). Intracellular adenosine is metabolized to inosine by adenosine deaminase or transformed into adenine nucleotides by adenosine kinase (10,12). Because of its rapid cellular uptake and metabolism, extracellular adenosine has a half-life of a few seconds (13), which can be prolonged by inhibition of its transport into cells (Fig. 1).

Several studies provide evidence that ticagrelor inhibits cellular uptake of adenosine (11,14,15). Ticagrelor inhibited adenosine uptake by washed human erythrocytes and by human, dog, and rat cell lines. Considering that the experiments were performed under sodium-free conditions and with cell lines that express ENT1 but not ENT2, it was assumed that ticagrelor inhibits sodium-independent ENT1 (14). The identity of the target transporter was recently confirmed with cells transfected with human transporters (ENT1, ENT2, CNT2, and CNT3). In these experiments, ticagrelor significantly inhibited adenosine uptake only in cells that expressed ENT1 (11). Compared with dipyridamole, an established ENT1 inhibitor (16), ticagrelor displayed a lower affinity for the transporter (K_i 41 vs. 2.6 nmol/l). Other $P2Y_{12}$ antagonists, (cangrelor, elinogrel, and the active metabolites of clopidogrel and prasugrel) did not display any significant activity versus any of the transporters. The main metabolites of ticagrelor (AR-C124910XX, present in blood, and AR-C133913XX, present in urine) showed weak ENT1 inhibition and low affinity (K_i: 330 and 23,000 nmol/l, respectively). Finally, the main metabolite of cangrelor displayed very weak inhibition of adenosine uptake (11). The 16-fold higher affinity of dipyridamole for ENT1 relative to ticagrelor is in line with the potency data obtained in the aforementioned in vitro experiments (14). The affinity of ticagrelor for $P2Y_{12}$ (K_i 2 nmol/l or pK_i 8.7) (17) is thus approximately 20-fold higher than for ENT1. Ticagrelor 1 μmol/l, but not the active metabolite of prasugrel, significantly conserved adenosine in human whole blood in vitro. Because the mean maximal plasma exposure after 90 mg of ticagrelor is 1.5 μmol/l (15), these in vitro data indicate a potential for clinical relevance. Importantly, it has been demonstrated that ticagrelor does not display relevant direct activity on adenosine receptors (11,18) and is not metabolized to adenosine (19).

Biological Effects of Adenosine

Adenosine exerts its biological effects by interacting with 4 G-protein–coupled receptors: A_1R and A_3R are coupled to G_i, the inhibitory G protein, which inhibits adenylyl cyclase and thus decreases intracellular cAMP, whereas $A_{2A}R$ and $A_{2B}R$ are coupled to the stimulatory G protein, G_s, which stimulates adenylyl cyclase, increasing intracellular cAMP. Of the 2 A_2R subtypes, A_{2A} is the high- and A_{2B} the low-affinity receptor (12,20).

Effects of adenosine on blood vessels. $A_{2A}R$ is the main adenosine receptor responsible for coronary vasodilation, which is mediated by both nitric oxide-dependent and -independent pathways (21). $A_{2B}R$ also mediates coronary vasodilation, whereas both A_1R and A_3R negatively modulate coronary vasodilation induced by $A_{2A}R$ and/or $A_{2B}R$ activation (22–24). Adenosine also induces endothelial progenitor cell migration via A_{2A} and A_3 (25).

Effects of adenosine on platelets. Adenosine is a potent inhibitor of platelet aggregation in platelet-rich plasma but not in whole blood as a consequence of its rapid uptake by erythrocytes. This discrepancy is abolished by the addition of dipyridamole to whole blood samples (26). Adenosine inhibits platelet activation mainly via $A_{2A}R$ but also via $A_{2B}R$ (27). The gene that encodes for A_{2B} is up-regulated after injury and systemic inflammation in vivo; as a consequence, the contribution of A_{2B} to adenosine-mediated platelet inhibition appears to increase under stress conditions (28).

Effects of adenosine on inflammatory responses. Adenosine modulates the inflammatory responses to a variety of stressful conditions. Low concentrations of adenosine, which activate A_1R and A_3R, promote neutrophil chemotaxis and phagocytosis, whereas high concentrations of adenosine, which activate $A_{2B}R$, inhibit neutrophil trafficking, granule release, and the production of reactive oxygen species and inflammatory mediators (29). The genetic deficiency of $A_{2B}R$ increased mortality in mice with sepsis and reduced levels of inflammatory markers (30). Thus, elevation of endogenous adenosine concentrations may reduce the inflammatory responses. Indeed, dipyridamole elevated adenosine concentrations and augmented the anti-inflammatory response during experimental human endotoxemia and was associated with a faster decline in proinflammatory cytokines (31).

Effects of adenosine on the heart. Adenosine exerts cardiac electrophysiological effects through activation of A_1R. It has a negative chronotropic effect through suppression of the automaticity of cardiac pacemakers and a negative dromotropic effect through inhibition of AV nodal conduction (32). These effects of adenosine constitute the rationale for its use as a diagnostic and therapeutic agent (e.g., treatment of supraventricular tachycardia) (33).