Arteriosclerosis, Thrombosis, and Vascular Biology

BRIEF REVIEW

P2X7 Receptors

An Untapped Target for the Management of Cardiovascular Disease

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ABSTRACT: Chronic low-grade inflammation contributes to the development of several diseases, including cardiovascular disease. Adequate strategies to target inflammation in cardiovascular disease are in their infancy and remain an avenue of great interest. The purinergic receptor P2X7 is a ubiquitously expressed receptor that predominately mediates inflammation and cellular death. P2X7 is a ligand-gated cation channel that is activated in response to high concentrations of extracellular ATP, triggering the assembly and activation of the NLRP3 (nuclear oligomerization domain like receptor family pyrin domain containing 3) inflammasome and subsequent release of proinflammatory cytokines IL (interleukin)-1β and IL-18. Increased P2X7 activation and IL-1β and IL-18 concentrations have been implicated in the development of many cardiovascular conditions including hypertension, atherosclerosis, ischemia/reperfusion injury, and heart failure. P2X7 receptor KO (knockout) mice exhibit a significant attenuation of the inflammatory response, which corresponds with reduced disease severity. P2X7 antagonism blunts blood pressure elevation in hypertension and progression of atherosclerosis in animal models. IL-1β and IL-18 inhibition has shown efficacy in clinical trials reducing major adverse cardiac events, including myocardial infarction, and heart failure. With several P2X7 antagonists available with proven safety margins, P2X7 antagonism could represent an untapped potential for therapeutic intervention in cardiovascular disorders.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cardiovascular diseases ■ heart failure ■ infant ■ inflammation ■ interleukins

Cardiovascular disease is the leading cause of mortality worldwide, representing up to 31% of annual global deaths.[1] Despite its widespread prevalence, there remain inadequate treatment options for a large proportion of patients, in part, due to the complex and varied pathophysiology involved in cardiovascular disease. In recent years, the role of inflammation in cardiovascular disease has been garnishing a lot of attention. Numerous studies have indicated a prominent role for low-grade inflammation in the development of hypertension, atherosclerosis, myocardial ischemic injury, and heart failure. Consequently, targeting the source of inflammation in these conditions remains a tantalizing yet elusive option. One such target that has shown promising results is the purinergic receptor P2X7.

P2RX7 (P2X7 receptor) belongs to a family of purinergic receptors divided into 2 classes: metabotropic G-protein–coupled P2Y receptors and ligand-gated ion channel P2X receptors. P2X receptors are primarily activated by extracellular ATP, with P2X7 being distinct from the other receptors due to its low affinity for ATP. P2X7 requires 100 to 1000× physiological concentrations of extracellular ATP for its activation with a reported EC_{50} (half maximal effective concentration) of ≈100 μmol/L.[2] With transient stimulation, the P2X7 receptor acts as a nonspecific cation channel facilitating Na^+ and Ca^{2+} influx and K^+ efflux, resulting in the activation of numerous downstream signaling complexes in a cell type–dependent manner.[3,4] The most prominent downstream effector of P2X7 activation is the NLRP3 (nuclear oligomerization domain like receptor family pyrin domain containing 3) inflammasome. The NLRP3 inflammasome cleaves and activates caspase-1, which subsequently cleaves the proinflammatory cytokines pro-IL (interleukin)-1β and pro-IL-18 into their mature, active forms. Prolonged stimulation of P2X7 with ATP promotes the formation of macropores in the cell membrane,

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Nonstandard Abbreviations and Acronyms

Ang II	angiotensin II
BP	blood pressure
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcome Study
I/R	ischemia-reperfusion
IL	interleukin
KO	knockout
MI	myocardial infarction
NLRP3	nuclear oligomerization domain like receptor family pyrin domain containing 3
TNF-α	tumor necrosis factor alpha
Treg	T regulatory cell

Highlights

- Accumulating evidence demonstrates that the purinergic receptor P2X7 plays a prominent role in chronic inflammatory conditions, including cardiovascular disease.
- P2X7 activation contributes to the development of hypertension through promotion of renal and vascular dysfunction.
- P2X7-mediated endothelial dysfunction and inflammation direct atherosclerotic plaque formation and rupture.
- In ischemic injury in the heart, P2X7 activation promotes cardiomyocyte death and enhances inflammation, leading to cardiovascular dysfunction.
- P2X7 inhibitors may provide a new avenue for treatment in cardiovascular disorders.

resulting in an inflammatory cell death program termed pyroptosis.[2,5–7] IL-1β and IL-18 are the primary mediators of P2X7-induced inflammation, which facilitate immune cell recruitment and inflammation, endothelial dysfunction, plaque formation, and cardiac dysfunction.[8–10]

Beyond the predominant role of P2X7 in triggering inflammation and cellular death, it has been implicated in numerous other functions including nociception, vascular function, glucose uptake, and paradoxically, promoting cellular survival (interested readers are directed to the following excellent articles[11–15]). The pleiotropic effect of P2X7 is in part cell type–dependent and in part dependent on the isoform of P2X7 expressed. Ten human splice variants have been identified, named P2X7A to P2X7J.[16–19] Isoform A (P2X7A)—the full length receptor—responds in a biphasic manner, with tonic activation by low concentrations of ATP promoting cellular proliferation and high concentrations of ATP promoting the typical responses of P2X7 activation, such as inflammasome activation and pore formation.[16,20,21] P2X7B has a truncated carboxy terminal, impairing its pore-forming ability.[20] It has been demonstrated to have an antiapoptotic effect in numerous cell types.[14,20–26] Isoforms P2X7C, P2X7E, P2X7G, and P2X7J also have a truncated carboxy terminal inhibiting their pore-forming ability, but their functional role is unclear, while the P2X7I isoform results in loss of function of the receptor.[16,19,27,28] In rodent T cells, an additional variant, P2X7K, mediates T-cell responses to ATP and NAD^+ (nicotinamide-adenine dinucleotide), facilitating T-cell class switching through CD62L (cluster of differentiation 62L) and CD27 cleavage and cellular death via externalization of phosphatidyl serine.[17,29–31] To date, a human homologue of P2X7K has yet to be identified, and its relevance in human pathology is unclear. Finally, P2X7 variants may be preferentially expressed in different cell types and are known to have varying affinities to ATP (P2X7K>A>B), which may help account for the cell type–dependent P2X7 responses.[17,18,21,32] Despite the work done to date to delineate the function of P2X7 variants, much remains unknown with regard to their role in disease progression, particularly in cardiovascular disease.

P2X7 receptor activation has been implicated in the progression of many chronic inflammatory diseases. In animal models, P2X7 inhibition has proven to be an effective treatment strategy for many chronic inflammatory disorders including arthritis, Duchenne muscular dystrophy, multiple sclerosis, Alzheimer disease, chronic pain, and cardiovascular disease.[33] However, the functional role of P2X7 outside of inflammation remains largely uncharacterized, and the pleiotropic nature of P2X7 function raises the question of the feasibility of P2X7 as a therapeutic target.

This review will focus on the role of P2X7 in the cardiovascular system and postulate on its utility as a target for treatment and management of cardiovascular conditions.

HYPERTENSION

Hypertension affects ≈1.13 billion people worldwide and is the largest cause of burden of disease worldwide and the most important risk factor for the development of cardiovascular disease.[1] Current studies investigating the role of P2X7 in hypertension are limited. However, available studies point to a role of P2X7 in regulating inflammation, as well as vascular and renal function in response to hypertensive challenges. The single-nucleotide polymorphism (rs598174) for P2X7 was strongly associated with both systolic and diastolic ambulatory blood pressure (BP) in a white population.[34] In a Chinese population of postmenopausal women, a hypomorphic single-nucleotide polymorphism (rs3751143) for P2X7 was associated with a decreased risk of primary (formerly called essential) hypertension.[35] Increased inflammasome expression and circulating IL-1β in subjects over the age of 60 years was strongly associated with increased risk for hypertension and vascular dysfunction,

as well as all-cause mortality.[36] Furthermore, elevated plasma ATP levels have been observed in hypertensive patients in comparison to normotensive controls or patients with controlled hypertension, leading to heightened T-cell responses in a P2X7-dependent manner.[37]

P2X7, IL-1β, AND HYPERTENSION

There is accumulating evidence that P2X7 contributes to the connection between low-grade chronic inflammation and hypertension.[8] Macrophages isolated from Dahl salt-sensitive rats produced more IL-1β in response to ATP than normotensive Lewis rats, highlighting heightened inflammasome responses in rats genetically predisposed to hypertension.[38] NLRP3 inflammasome proteins in mice and IL-1β in humans have been reported to be elevated in hypertension, and directly antagonizing the NLRP3 inflammasome has modestly reduced BP in various animal models of hypertension.[39–42] Targeting IL-1β using anakinra—an IL-1 receptor antagonist—significantly reduced BP in a 1-kidney deoxycorticosterone acetate–salt model of hypertension.[43] However, the efficacy of targeting IL-1β in human hypertensive patients is unclear. In CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study), patients given an anti-IL-1β monoclonal antibody (canakinumab) had no reduction in BP at 3, 6, or 12 months of follow-up, and there was no reduction in incident hypertension in the cohort.[44,45] Despite a lack in reduction of BP, patients with elevated systolic BP (130–140 mm Hg) or hypertension (systolic BP, >140 mm Hg) treated with canakinumab had a significant reduction in composite end points (myocardial infarction [MI], stroke, or cardiovascular mortality).[44,45] The CANTOS trial suggests that blocking IL-1β alone may be insufficient to reduce BP, and, therefore, targeting upstream of IL-1β, at the P2X7 receptor, may provide a more attractive target for BP management as it could antagonize additional downstream effects of P2X7 activation outside of IL-1β production (discussed below).

P2X7 AND KIDNEY FUNCTION

Under nonpathological conditions, there is sparse P2X7 expression throughout the kidney. However, P2X7 expression is significantly increased in hypertensive states.[46] Transgenic rats expressing the mouse *Ren2* gene have an overactivated renin-angiotensin-aldosterone system and develop severe hypertension that can be attenuated with angiotensin-converting enzyme inhibitors.[47,48] These transgenic rats have increased P2X7 expression in the glomeruli in comparison to normotensive rats.[46] Other hypertensive models demonstrate similar results, with P2X7 expression significantly increased in the kidney in Ang II (angiotensin II) and deoxycorticosterone acetate–salt-induced hypertensive rodents, as well as in Dahl salt-sensitive rats.[38,49–51] P2X7 receptor silencing decreased renin activity and angiotensin-converting enzyme 1 and 2 expression in the renal cortex, preventing renal dysfunction in a model of diabetic nephropathy.[52] In addition, P2X7 antagonism may also reduce the prohypertensive effects of Ang II. Ang II acts as a potent vasoconstrictor of the renal vasculature, and it can alter renal sodium and water handling through increased aldosterone release.[53] In rodent models, P2X7 antagonism reduced renal vascular resistance and increased medullary perfusion, resulting in enhanced pressure natriuresis.[49,50,54] Menzies et al[49] reported a 6-fold increase in sodium excretion with P2X7 antagonism, blunting Ang II–induced BP elevation in rats. In addition, ATP promotes transepithelial sodium transport through epithelial sodium channels, which can be attenuated by Brilliant Blue G—a P2X7 antagonist.[55] This, along with increased pressure natriuresis, may account for the increased Na^+ excretion associated with P2X7 antagonism.[49,50] However, another study found that P2X7 antagonism had no effect on Ang II–induced BP elevation in rats, although the authors used a 10-fold higher dose of Ang II, which may account for the differences observed.[50] Overall, these studies provide evidence for a role of P2X7 in the regulation of kidney responses to hypertensive stimuli and support P2X7 as a novel antihypertensive target.

Further supporting the beneficial effects of inhibiting P2X7, activation of the receptor itself exerts prohypertensive effects in the kidney. Ang II and aldosterone both increase renal ATP concentrations, with the concentration of renal interstitial ATP strongly correlated with BP increase.[56,57] P2X7 activation on the renal vasculature, by elevated ATP, appears to exert a tonic vasoconstrictive effect.[49] In addition, P2X7-mediated vasoconstriction of the medullary microcirculation has been shown to cause regional hypoxia promoting vascular hypertrophy and renal inflammation.[49] Prolonged exposure to elevated extracellular ATP results in P2X7-mediated mesangial, fibroblast, endothelial, and renal tubular cell death, contributing to renal inflammation and fibrosis, as well as promoting endothelial dysfunction.[58–62] P2X7 antagonism results in a partially NO-dependent vasodilation of the afferent, efferent, and renal arteries, increasing renal perfusion and reducing renal inflammation and fibrosis.[49,50,52,54] P2X7 KO (knockout) or antagonism has also proved effective in preventing renal fibrosis, renal immune cell infiltration, and lowering BP and albuminuria in Dahl salt-sensitive rats and in a deoxycorticosterone acetate–salt model of hypertension.[38,51] In summary, continuous P2X7 activation leads to microvascular dysfunction and regional hypoxia. This promotes renal inflammation and renal fibrosis, leading to a decline in renal function that contributes to hypertension.

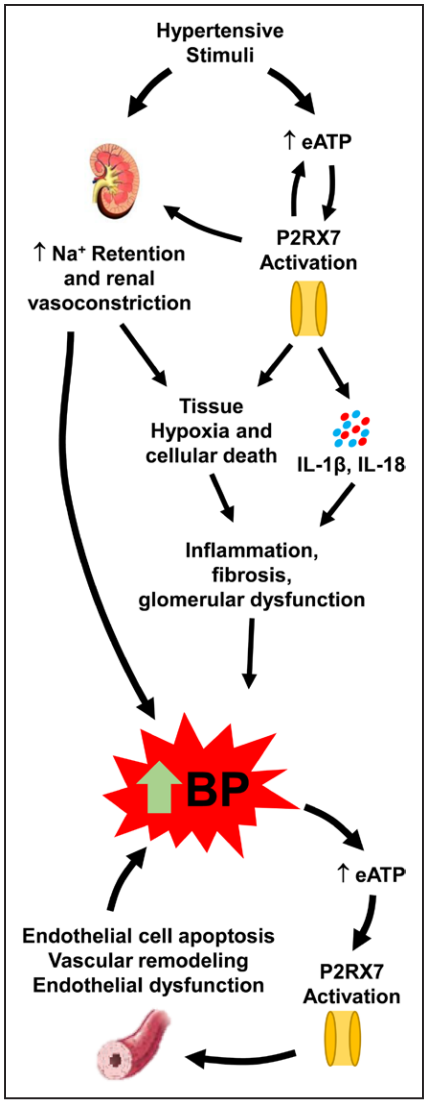

Figure 1. P2X7 and hypertension.
Hypertensive stimuli induce an upregulation of P2RX7 (P2X7 receptor) surface expression,[46,49,50] as well as directly and indirectly cause increases in extracellular ATP (eATP) in the renal interstitial fluid.[56,57] Elevated ATP activates P2RX7 promoting cellular death, causing the release of proinflammatory cytokines, inducing renal vasoconstriction, and promoting sodium retention.[49] P2X7-induced renal vasoconstriction causes tissue hypoxia, where along with inflammatory cytokines and reactive oxygen species, it causes inflammation, fibrosis, and glomerular dysfunction.[49,58–62] Together, renal fibrosis, increased sodium retention, and renal vasoconstriction promote a rise in blood pressure (BP) that can increase systemic circulating ATP concentrations. The resulting P2X7 activation promotes endothelial cell apoptosis,[67,68] vascular remodeling, and ultimately endothelial dysfunction,[62,71] which further exacerbates the increase in BP. IL indicates interleukin.

P2X7 AND SYSTEMIC VASCULATURE

P2X7 expression has been reported in the endothelium and the smooth muscle layer of most of the systemic arterial and venous circulation in human and animal tissues.[63–66] In the microvasculature, P2X7 activation has been shown to promote vascular dysfunction through increased oxidative stress and increased endothelial cell permeability and apoptosis. In a rat model of type 1 diabetes, P2X7 expression was found to be elevated in the retinal microvasculature, contributing to increased microvasculature permeability, whereas in human retinal endothelial cells, P2X7 activation induced endothelial cell death.[67,68] In both experiments, microvasculature dysfunction could be reversed by a P2X7 inhibitor. Further, it was demonstrated that P2X7 vasotoxicity was mediated through P2X7-dependent pore formation, as well as NADPH (reduced nicotinamide-adenine dinucleotide phosphate) oxidase–dependent ROS generation.[69] In addition, surgical stretch of human saphenous veins prepared for coronary artery bypass grafts caused P2X7 activation inducing apoptosis resulting in vascular dysfunction.[60] P2X7 activation can also induce constriction of the retinal and renal microvasculature, as well as of large veins, which could lead to increased systemic vascular resistance.[49,50,63,70] In diabetic rats, P2X7 antagonism improved endothelium-dependent relaxation and decreased constrictor responses to phenylephrine in the aorta.[71] A model investigating vascular surgical stretch injury demonstrated that P2X7 activation diminished endothelium-dependent relaxation through decreased NO production.[62] The resulting vascular dysfunction and remodeling can contribute to increased systemic vascular resistance and the development of hypertension.[72,73]

However, conflicting results suggest P2X7 activation may also play a role in vasodilatation. P2X7 activation on murine mesenteric artery endothelial cells resulted in enhanced NO production.[74] In addition, P2X7-mediated responses to lipopolysaccharides have been reported to cause hyporeactivity of the thoracic aorta in mice, leading to P2X7-mediated hypotension in an IL-1β–dependent and NO-dependent manner.[75,76] These studies highlight critical differences in the role of P2X7 responses in different tissues and different disease states. It remains unclear whether, in a chronic disease setting such as hypertension, P2X7 antagonism could be beneficial or detrimental to vascular function and mechanics, and the area warrants further investigation.

ATHEROSCLEROSIS

Atherosclerosis is a common comorbidity with hypertension and presents similar features, such as endothelial dysfunction and low-grade inflammation.[77] Immune cell recruitment and activation at the site of plaques is required for the development of atherosclerotic lesions, and P2X7-directed inflammation could play a central role in plaque formation and promoting plaque rupture. In human carotid arteries presenting with atherosclerotic plaques, there is increased P2X7 expression in plaque-rich areas compared with regions devoid of plaques.[66,78] In addition, the expression of P2X7 mRNA in circulating mononuclear cells significantly correlates with the

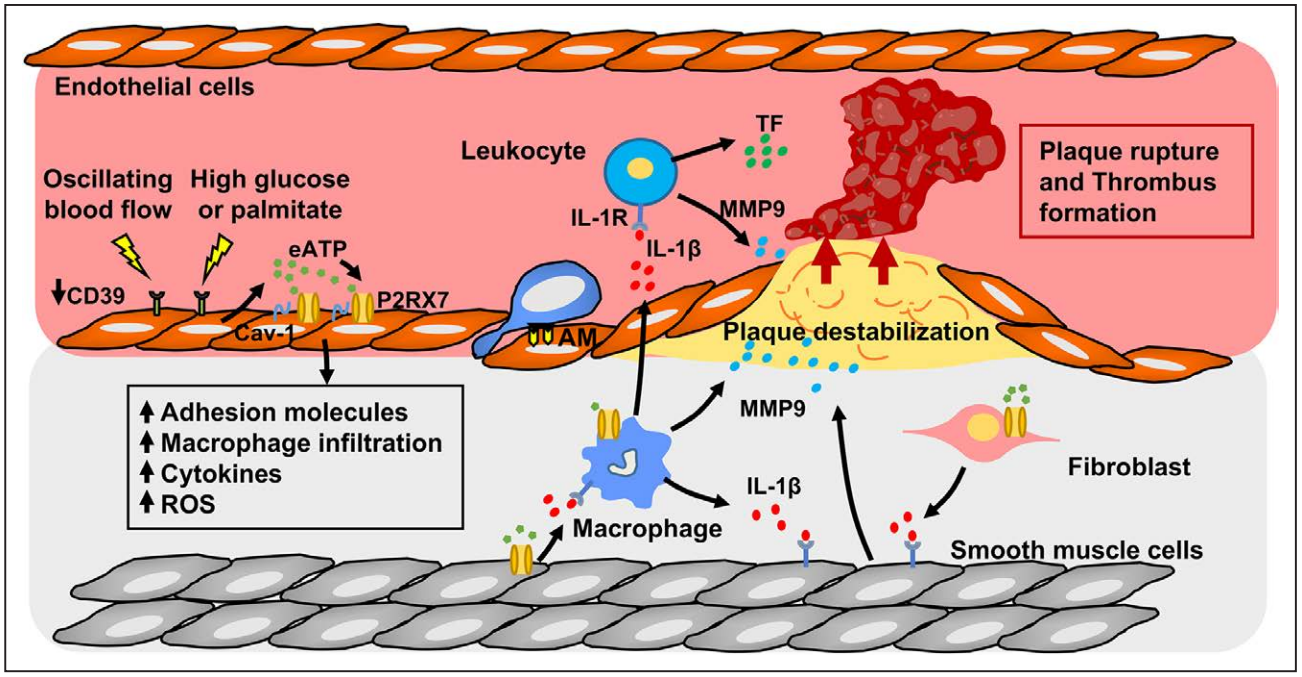

Figure 2. P2X7 and atherosclerosis.
Oscillating flow or high glucose or palmitate promote P2RX7 (P2X7 receptor) surface expression,[87] elevate extracellular ATP (eATP),[82,83] and decrease CD39 (cluster of differentiation 39) expression[84,85] in the endothelium at sites prone to develop atherosclerosis, creating an environment suitable for enhanced P2X7 activation. Endothelial P2X7 activation promotes leukocyte recruitment, adhesion, and transmigration into the developing plaque through production of inflammatory cytokines and increased adhesion molecule (AM) expression on endothelial cells.[78,80,87,93] P2X7-dependent IL (interleukin)-1β production from vascular smooth muscle cells (VSMCs), macrophages, and fibroblasts promotes MMP9 (matrix metalloprotease 9) release from macrophages and VSMCs.[80,102–104] MMP9 destabilizes the plaque, making it vulnerable to rupture,[80,104] whereas P2X7 activation on myeloid cells induces the release of TF (tissue factor) promoting thrombus formation.[105,106] Cav-1 indicates caveolin-1; IL-1R, interleukin-1 receptor; and ROS, reactive oxygen species.

degree of coronary artery stenosis.[79] ATP accumulates in atherosclerotic vessels as compared with nonatherosclerotic ones, and elements of the inflammasome (NLRP3, caspase-1, and IL-1β) are increased in plaque-rich regions, providing an indication of P2X7 activation.[66,80,81] Together, these studies provide support for an involvement of P2X7 in the development of atherosclerosis, and there are several potential mechanisms for P2X7 activation in atherosclerosis.

P2X7 activation in atherosclerosis may be initiated through alterations in blood flow (turbulent blood flow) or as a result of a secondary metabolic disorder. At sites with turbulent blood flow, there is a dramatic elevation in local extracellular ATP.[82,83] The increase in ATP is driven through decreased ATPase (CD39) expression and an enhanced release of ATP from endothelial cells in regions rich with caveolin.[84–86] These sites of turbulent blood flow have increased P2X7 expression, which can colocalize with caveolin-1, placing P2X7 receptors proximal to sites of ATP release.[87–91] These P2X7 receptors expressed in caveolin-1–rich domains have been shown to be nonpore forming and instead facilitate intracellular Ca^{2+} accumulation, leading to p38 mitogen-activated protein kinase phosphorylation and subsequent upregulation of surface adhesion molecules in plaque prone regions.[87,88,92] In addition, exposure of endothelial cells and human fibroblasts to high concentrations of glucose or palmitate, such as in diabetes, causes extracellular ATP release and the formation of P2X7 aggregates near the cell periphery.[93–95] These P2X7 aggregates have a lowered threshold for activation to ATP and mediate endothelial dysfunction through elevated ROS generation, increased cell permeability, and expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.[78,93,94,96,97] Furthermore, oxidized low-density lipoproteins and cholesterol crystals, common elements in atherosclerosis, can activate the NLRP3 inflammasome resulting in the release of IL-1β and IL-18.[98–101] Consequently, factors common to atherosclerosis development, hyperglycemia, hyperlipidemia, oxidized low-density lipoproteins, cholesterol crystals, and turbulent blood flow have been shown to influence P2X7 activation.

P2X7 activation on endothelial cells at sites prone to development of atherosclerosis promotes leukocyte recruitment, adhesion, and transmigration into the developing plaque through the production of proinflammatory cytokines, ROS generation, and increased cellular adhesion molecules on endothelial cells.[78,80,87,93] The subsequent tissue damage amplifies extracellular ATP concentrations and facilitates P2X7-mediated IL-1β secretion from smooth muscle cells and infiltrating leukocytes.[78,80,102] Secreted IL-β then triggers

the release of matrix metalloprotease 9 from vascular smooth muscle cells and leukocytes, which destabilizes the plaque and renders it vulnerable and prone to rupture.[80,102–104] Furthermore, P2X7 facilitates thrombosis at the site of the ruptured plaque. When exposed to elevated circulating ATP, myeloid and smooth muscle cells release tissue factor in a P2X7/ROS-dependent manner, which triggers thrombus formation, and can lead to coronary obstruction and sudden death.[105,106]

Strategies targeting P2X7 or its downstream effectors have proven efficacious in preventing atherosclerosis progression in several preclinical and clinical models. P2X7 KO mice present with lower blood cholesterol than wild-type mice and in atherosclerotic animal models have decreased plaque size.[78,107] The reduction in lesion size appears to be the result of decreased leukocyte recruitment and macrophage infiltration in P2X7 KO animals or after P2X7 antagonism.[78,87] The attenuated immune infiltration was associated with decreased adhesion molecule expression on endothelial cells, with decreased caspase-1 activation and proinflammatory cytokine release.[78,87] Decreased cholesterol levels in P2X7 KO mice may also play a role in decreasing inflammation, as oxidized low-density lipoproteins and cholesterol crystals have been shown to induce inflammasome activation that promotes atherosclerosis.[98,99,101,107] In addition, P2X7 receptor targeting or IL-1β blockade increased plaque stability through inhibition of matrix metalloprotease 9 release.[80,104] In the CANTOS trial, IL-1β blockade resulted in a reduction in all cardiovascular events, including coronary revascularization and MI, without lowering systemic lipid levels.[44] This reduction in adverse cardiovascular events was comparable to the effects of lipid lowering by proprotein convertase subtilisin-kexin type 9 inhibitors.[44,108,109] Whether P2X7 antagonism rather than P2X7 KO also reduces blood cholesterol has yet to be determined. In summary, targeting downstream P2X7 effector molecules or P2X7 receptors prevents leukocyte recruitment and inflammation in plaques, prevents plaque rupture, and may have lipid-lowering and antithrombotic effects, making P2X7 a potential target in managing atherosclerosis.

HEART DISEASE

Heart disease encompasses a wide variety of conditions with inflammation being the primary driver of many noncongenital conditions.[110] IL-1β and IL-18, downstream effectors of P2X7, have been repeatedly identified as mediators to this inflammatory response.[111,112] A loss-of-function P2X7 variant rs3751143 was significantly associated with a decreased risk of ischemic heart disease and stroke, especially in individuals with hypertension.[113] However, the contribution of P2X7 to heart disease has still yet to be fully elucidated.

MYOCARDIAL ISCHEMIC INJURY

During cardiac ischemia, there is an interruption of blood flow to coronary tissue that can disrupt cardiac function and damage surrounding tissues, resulting in a substantial release of ATP.[114,115] The rise of ATP following ischemia/reperfusion (I/R) activates surrounding cardiac fibroblasts, stimulating P2X7-mediated release of IL-1β, IL-18, and ROS that can lead to the recruitment of leukocytes to the hypoxic region.[116–118] The recruited leukocytes then contribute to amplify inflammation through P2X7-mediated activation and release of IL-1β and IL-18, thus promoting myocardial damage and cardiac fibrosis leading to declining cardiac function.[118–120] Inhibition of IL-1β, IL-18, or caspase-1 significantly decreased infarct size and improved contractile function of the heart.[118,119] However, whether P2X7 antagonism alone in I/R in the heart would also be protective is unclear.

Paradoxically, preconditioning cardiac tissue with short bouts of I/R has shown to protect from I/R injury through an ATP-driven mechanism.[121] Cardiac protection was facilitated through the release of sphingosine-l-phosphate and adenosine via P2X7/pannexin-1 pores, occurring pre-ischemia and post-reperfusion.[121–123] Inhibition of pannexin-1 or P2X7 abrogated the protective effect of I/R conditioning and resulted in increased infarct sizes.[121] The difference between protection and harm associated with P2X7 activation may be the result of P2X7 splice variants. Splice variants of P2X7 are known to have varying affinities for ATP and can elicit different responses.[17] Further strengthening this hypothesis, P2X7 functional coupling with pannexin-1 was found to be dependent on the P2X7 isoform expressed, specifically to a common allelic mutation resulting in a proline-to-leucine mutation at amino acid 451 in the P2X7A variant.[17] This same mutation was found to result in a decreased sensitivity to ATP (≈10-fold).[124] In addition, activation of P2X7A with low concentrations of ATP has been demonstrated to have growth-promoting effects.[21] Since P2X7-mediated protection from I/R was dependent on pannexin-1 coupling, it is possible that the differing effects of P2X7 in I/R are dependent on the isoform of P2X7 expressed. Whether the protective effect of P2X7 activation during I/R is mediated through one of these splice variants has yet to be shown, but if this is the case, this could provide a selective target to protect the heart during I/R without the accompanying inflammation.

ANGINA PECTORIS

Angina is a common symptom in many patients experiencing coronary ischemia, and P2X7 appears to play an important role in persistent angina symptoms post-MI. After acute MI, P2X7 mRNA and protein were upregulated in the superior cervical ganglia and in cardiac

sympathetic afferents of rats.[125–127] P2X7-dependent transmission of nociception down these cardiac afferents has been demonstrated, along with activated cardiac sympathetic efferent nerves, leading to increased BP, heart rate, and circulating proinflammatory cytokines (TNF-α [tumor necrosis factor alpha] and IL-6). P2X7 antagonism post-MI attenuates sympathetic stimulation of cardiac tissue, reducing tachycardia, BP, myocardial injury, and nociception signaling, ultimately alleviating symptoms of angina.

MI AND HEART FAILURE

MI is a life-threatening condition caused by obstruction of blood flow to cardiac tissue. Following an acute MI, there is a substantial increase in extracellular ATP released from damaged cells. This rising extracellular ATP promotes P2X7-mediated inflammasome formation and activation around the border of the infarct in surrounding fibroblasts, cardiomyocytes, and invading leukocytes, leading to elevated IL-1β and IL-18.[128–130] P2X7 activation in cardiomyocytes promotes caspase-dependent apoptosis, which contributes to cardiac dysfunction.[128,131,132] During acute MI, epicardium-derived cells are also directed to the infarct region.[120] Epicardium-derived cells give rise to various cardiovascular cells and migrate to injured myocardium to initiate tissue repair.[133–136] However, during ischemia, invading epicardium-derived cells can also promote further inflammation by secreting ATP, NAD, and tenascin-C.[120] Tenascin-C can prime the NLRP3 inflammasome via toll-like receptor 4 activation and coupled with elevated ATP, can activate the inflammasome in infiltrating leukocytes, further amplifying inflammation.[118,120] Elevated IL-1β and IL-18 contribute to cardiac enlargement, cardiac fibrosis, and a deterioration of heart function post-MI leading to heart failure.[119,128,137,138] Additionally, the NAD released by epicardium-derived cells can cause P2X7-mediated phosphatidyl serine exposure on the outer leaflet of T regulatory cells (Tregs) leading to their death.[29,30,139,140] Tregs normally increase in ischemic tissue 3 to 7 days after reperfusion and contribute to resolution of inflammation and promote tissue repair.[141] P2X7 activation may lead to a decreased presence of anti-inflammatory Tregs in ischemic tissue, and indeed, P2X7 antagonism in a kidney I/R model resulted in a significant increase of infiltrating Tregs and improved tissue recovery.[142]

Antagonizing or knocking out P2X7 or its downstream effectors, caspase-1 or NLRP3, in animal models decreased infarct size, improved cardiac function, and enhanced survival post-MI via reduced IL-1β and IL-18 levels in the heart.[128,130,137,143] Targeting IL-1β directly has also proven effective in reducing cardiac dysfunction and promoting survival post-MI in animal models and in clinical trials.[44,138,144,145] The protective effect of P2X7 antagonism in ischemia and acute MI appears to be due to decreased inflammation through decreased proinflammatory cytokine production and increased anti-inflammatory Tregs. Therefore, targeting P2X7-mediated inflammation post-MI may provide a therapeutic avenue for improved cardiac function and survival in patients. Indeed, circulating P2X7 mRNA expression is predictive of prognosis in acute MI, with elevated P2X7 expression correlating with worse patient outcomes.[79]

CEREBRAL ISCHEMIC INJURY

P2X7 activation has also been implicated in cerebral ischemic injury (ischemic stroke). In a permanent focal cerebral ischemia model, P2X7 expression was upregulated on neuronal and glial cells post-ischemia and was particularly associated with apoptotic cells.[146] P2X7 antagonism in rat transient focal cerebral ischemia models resulted in decreased infarct size and neuronal death and improved survival.[147,148] Interestingly, a protective effect in P2X7 KO mice has not been demonstrated. Le Feuvre et al[149] saw no improvement in infarct volume 24 hours after inducing temporary cerebral ischemia in P2X7 KO mice but did see an improvement using an IL-1 receptor antagonist. In an acute ischemic stroke model in mice, P2X7 KO led to larger edema size within the first 24 hours of reperfusion but not after 72 hours.[150] It is possible that P2X7 activation on microglia by low concentrations of ATP after cerebral I/R provides neuroprotection,[150–152] while prolonged stimulation of P2X7 on glial and neural cells results in cellular death and inflammation.[147,148,153] Therefore, P2X7 appears to be a double-edged sword in cerebral I/R injury, with P2X7 activation initially providing a neuroprotective benefit, but with prolonged activation shifting to become a proinflammatory mediator exaggerating cerebral ischemic injury.

THERAPEUTIC POTENTIAL OF P2X7 INTERVENTION

Downstream targets of P2X7 activation, mainly IL-1β, have been investigated in several clinical studies for efficacy in managing cardiovascular disease and have yielded promising results. The CANTOS trial using the IL-1β antagonist canakinumab was one of the first trials to demonstrate that the risk for recurrent cardiovascular disease could be decreased by lowering inflammation without lowering systemic lipid levels.[44] However, patients on canakinumab had a significantly increased risk of fatal infection, although there was no difference in all-cause mortality between groups (median patient follow-up of ≈3.7 years). Targeting P2X7 rather than IL-1β could have several advantages. First, P2X7 activation is a major mediator of IL-1β production but not the only one, and whether prolonged use of a P2X7 antagonist would also increase the risk of fatal infection

is unclear at this time, although clinical trials conducted thus far with P2X7 antagonists have had limited-to-no serious adverse advents reported for up to 6 months of treatment. Additionally, P2X7 antagonism has the added benefit of blocking other downstream effects of P2X7 activation that can be deleterious to health, such as cellular death. Finally, P2X7 antagonism may be especially beneficial in patients with cardiovascular disease and metabolic disorders such as hyperlipidemia or hyperglycemia. In preclinical models, P2X7 antagonism was able to diminish inflammasome activation by non-nucleotide agonists such as oxidized low-density lipoproteins, glucose, and palmitate, highlighting an additional benefit when treating disorders such as atherosclerosis.[93,101]

Although animal models targeting P2X7 in cardiovascular disease have shown favorable results, to date, there have been no clinical trials investigating P2X7 antagonism in cardiovascular disease. Over 70 patents for P2X7 antagonists have been filed, with several P2X7 antagonists having undergone clinical investigation for various inflammatory conditions with mixed results (Table).[154] AstraZeneca P2X7 antagonist (AZD9056) had no effect on reduction of inflammatory biomarkers or disease index in patients with chronic obstructive pulmonary disease or rheumatoid arthritis but modestly improved the disease index in Crohn disease (specifically decreased nociception) despite no reduction in inflammatory biomarkers.[155–157] Similarly, Pfizer P2X7 antagonist (CE-224535) was inefficacious in lowering disease activity or inflammatory biomarkers in rheumatoid arthritis patients inadequately controlled by methotrexate.[158] Ex vivo analysis had demonstrated that AZD9056 was able to inhibit IL-1β ex vivo in human monocytes, and, therefore, it was postulated that in these pathologies, inhibiting the P2X7-IL-1β and IL-18 inflammatory axis was insufficient to control disease progression and that other inflammatory cytokines could potentially be major contributors.[156] Due to the failure of these drugs to adequately suppress systemic inflammation, both companies abandoned their clinical trials after completion of phase II.[155–158] Recently, a phase II clinical trial by Evotec and Second Genome investigating P2X7 antagonism in nonalcoholic steatohepatitis was also terminated due to an unfavorable risk-benefit profile with their P2X7 inhibitor.[159]

Despite underwhelming results from early clinical trials, the recent crystallization of P2X7 has further facilitated the development of more targeted P2X7

Table. Current and Past Clinical Trials Investigating the Efficacy of P2X7 Antagonism for Disease Management

Company	Compound name	Indication	Phase	Study centers	Year completed	No. of patients enrolled (completed)	Results	Reference
AstraZeneca	AZD9056	Chronic obstructive pulmonary disease	II	28 centers across Bulgaria, Germany, Hungary, Sweden, and the United Kingdom	2006	271 (120)	Safe, tolerable, no effect on lung function	155
		Crohn disease	II	10 centers across Belgium, France, Germany, Austria, and Hungary	2007	34 (30)	Safe, tolerable, improvement in Crohn Disease Activity Index, no decrease in inflammatory markers (CRP)	157
		Rheumatoid arthritis	II	51 centers across Argentina, Australia, Belgium, Canada, Czech Republic, France, Mexico, Poland, Romania, Russian Federation, Slovakia, and the United States	2009	385 (316)	Safe, tolerable, no improvement in disease	156
Biosceptre	nfP2X7 antibody	Basal cell carcinoma	I	3 sites across the United States	2014	21 (20)	Safe, tolerable, reduction in lesion size	160
Evotec/Second Genome	SGM 1019	Nonalcoholic steatohepatitis	II	10 sites across the United States	2019	9	Phase II terminated due to unfavorable risk-benefit profile	159
GlaxoSmith-Kline	GSK1482160	Inflammatory pain	I	1 center in the United Kingdom	2009	10 (10)	Not possible to achieve level of pharmacology (>90% IL-1b inhibtion) within an adequate safety margin	161
Pfizer	CE-224535	Rheumatoid arthritis	II	24 centers across Chile, Czech Republic, Mexico, Poland, Republic of Korea, Spain, and the United States	2009	100 (86)	Safe, tolerable, no improvement in disease condition	158
Janssen	JNJ-54175446	Mood disorders	II	5 centers across the United Kingdom	Underway	142*	Recruitment suspended due to COVID-19 pandemic	162,163

COVID-19 indicates coronavirus disease 2019; CRP, C-reactive protein; and IL, interleukin.
*Estimated patient recruitment number.

antagonist therapeutic strategies that could further enhance clinical efficacy.[164] Janssen has designed new P2X7 agents for diagnosis and treatment of mood disorders that can penetrate the blood-brain barrier and have shown encouraging results in phase I clinical trials.[162,163,165] Specific interest has begun to emerge at targeting P2X7 variants in disease settings. Biosceptre has developed a monoclonal antibody to an epitope termed E200, which is associated with nonfunctional variants of P2X7 and has demonstrated efficacy in a phase I clinical trial for the treatment of basal cell carcinoma.[160] As P2X7 variants may also contribute to the pathogenesis of cardiovascular disease, such as I/R injury, it is an interesting avenue of research that merits more attention. Recently, a P2X7-specific nanobody, one-tenth the size of an antibody, was developed that was able to block P2X7-mediated IL-1β release with 1000× greater potency than Janssen or AstraZeneca small molecule inhibitors JNJ47965567 and AZ10606120.[166,167] The enhanced specificity of P2X7 antagonists opens the door for potentially targeting other P2X7 variants in disease settings and will be an interesting avenue of research to follow over the coming years.

Despite the lack of efficacy for disease management of early P2X7 antagonists in human clinical trials, they provide evidence for the relative tolerability of P2X7 antagonists, as limited-to-no serious adverse advents were reported in the majority of clinical trials conducted to date. Therefore, since animal models have demonstrated a potential benefit for P2X7 antagonism in the context of hypertension, atherosclerosis, and heart disease and clinical trials have provided a precedent for safety of P2X7-directed inhibitors, P2X7 antagonists may represent a viable therapeutic option in the management of cardiovascular disease.

CONCLUSIONS

P2X7 is a key player in promoting inflammatory responses to tissue injury. In cardiovascular disease, P2X7 activation promotes endothelial dysfunction and inflammation that drives kidney and cardiac dysfunction, atherosclerosis, hypertension development, and the progression of heart failure. Preclinical models investigating P2X7 receptor KO or antagonism in cardiovascular disease have shown promising results in attenuating disease. Current clinical trials of P2X7 antagonists have shown P2X7 antagonists to be mostly well tolerated, and, therefore, P2X7 inhibition may represent an untapped resource for the management of cardiovascular disease.

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