Roles of Prostaglandin E2 in Cardiovascular Diseases

Focus on the Potential Use of a Novel Selective EP4 Receptor Agonist

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SUMMARY

Prostaglandin E2 (PGE₂) is produced in inflammatory responses and regulates a variety of immunological reactions through 4 different receptor subtypes; EP1, 2, 3 and 4. However, the precise role of each receptor in cardiovascular disease has not yet been elucidated. Enhanced expression of some EPs has been observed in clinical and experimental cardiovascular diseases. EP agonists have been developed to clarify the role of each receptor. Recently, we developed a novel selective agonist to examine the effects of EP4 on cardiac transplantation, myocardial ischemia, and myocarditis. Of note, a selective EP4 agonist attenuated inflammatory cytokines and chemokines via attenuation of macrophage activation in inflammatory heart diseases. In this review article, we discuss the effects of PGE₂ receptor agonists on the development of cardiovascular diseases. (Int Heart J 2011; 52: 266-269)

Key words: Prostaglandin, Receptors, Chemokine, Macrophage, Heart

Prostanoids are known to be multifunctional physiologic factors. They are produced by various stimuli and participate in local homeostasis and physiologic responses. They are synthesized from arachidonic acid and quickly released to the extracellular medium to send signals via prostanoid receptors.¹⁾ The prostanoid receptors, DP, EP, FP, IP and TP, are bound to PGD₂, PGE₂, PGF_{2α}, PGI₂, and TXA₂, respectively.²⁾ In the cardiovascular system, these receptors are expressed on various cells, such as cardiomyocytes, vascular endothelium and smooth muscle cells.³⁾ They play an important role in modulating homeostasis in the development of cardiovascular diseases, such as thrombosis and atherosclerosis.⁴⁾ Recent studies revealed that prostanoids play an important role in both the occurrence and suppression of various cardiovascular diseases, such as myocardial ischemia, cardiac hypertrophy, cardiac fibrosis, and atherosclerosis.⁵⁾ However, the detailed roles of prostanoids in cardiovascular disease have not yet been completely elucidated. To clarify the mechanisms, selective receptor agonists have been developed.

Among the prostanoids, PGE₂ is known to play a key role in the pathogenesis of cardiovascular diseases. It was reported that a deficiency of PGE₂ synthase-1 reduced plaque burden in fat-diet low-density lipoprotein receptor knockout mice.⁶⁾ Another report revealed that PGE₂ synthase-1 knockout mice showed impaired left ventricular contraction after acute myocardial infarction.⁷⁾ These studies indicate that PGE₂ plays a pivotal role in cardiovascular inflammation.^{8, 9)} PGE₂ exerts their effects via G-protein-coupled receptors, and PGE₂-medi-

ated cardioprotection is involved in the EP receptor subtypes.¹⁰⁾ Each receptor has their unique signaling pathways. EP1 couples to Gq and increases intracellular Ca²⁺. EP3 couples to Gi and inhibits the increase of cAMP. EP2 and EP4 receptors are coupled to stimulate adenylate cyclase, which leads to the elevation of intracellular cAMP.¹¹⁾ In these EP receptors, EP3 and EP4 are known to have cardioprotective effects in various *in vitro* and *in vivo* models. Recently, it was reported that PGE₂ suppressed production of chemokines, such as monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α, in human macrophages via EP4.¹²⁾ The activation of EP4 also inhibited the production of proinflammatory cytokines¹³⁾ and adhesion molecules.¹⁴⁾ These studies suggest that EPs play a pivotal role in anti-inflammatory mechanisms. In this article, we review the recent progress of EP agonists and their role in inflammation-related cardiovascular diseases.

SYNTHESIZED EP AGONISTS

i) EP1 agonists: ONO-DI-004 and 17-phenyl-trinor-PGE₂ are well known as selective EP1 agonists (Figures 1 A and B).¹⁵⁻¹⁷⁾ Mizuguchi, *et al* revealed that myoelectrical activity was suppressed by continuous infusion of ONO-DI-004 into the gastric artery. This demonstrates that EP1 signaling has a crucial role in the suppression of myoelectrical activity of gastric smooth muscles and inhibition of gastric emptying.¹⁸⁾ Exogenous application of 17-phenyl-trinor-PGE₂ elicited arteriolar

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Figure 1. Chemical structures of the compounds.

constrictions which were enhanced in db/db mice.¹⁹⁾

ii) EP₂ agonists: ONO-AE1-259, CP-533536, 19(*R*)-hydroxy PGE₂, AH13205 and misoprostol are known as major EP₂ agonists.^{16,17)} Among them, ONO-AE1-259 has been widely used to clarify the roles of EP₂ (Figure 1C). This compound reduced the infarct volume correlated with improved neurologic scores in mice.²⁰⁾ It also regulated ocular hemodynamics in rats.²¹⁾ The compound induced beta1-integrins in human coronary arterial endothelial cells.²²⁾ Another EP₂ agonist, CP-533536, healed fractures when administered locally as a single dose in rat models of fracture healing.²³⁾ Other compounds, 19 (*R*)-hydroxy PGE₂²⁴⁾ and AH13205, also act as selective EP₂ agonists which sprouted nerve fibers.²⁵⁾ It is known that misoprostol has a significant protective effect in cerebral ischemia via EP₂ receptor activation.²⁶⁾

iii) EP₃ agonists: ONO-AE-248, sulprostone, SC-46275, MB-28767, GR-63799, and misoprostol are known as major EP₃ agonists.^{16,17)} In this group, ONO-AE-248 and sulprostone have been investigated to clarify the roles of EP₃ (Figures 1D and E). Recently, it was reported that ONO-AE-248 augmented glutamate-induced excitotoxicity in CA1 neurons.²⁷⁾ It also suppressed murine contact hypersensitivity with suppression of neutrophil-recruiting chemokines.²⁸⁾ Sulprostone is known to stimulate duodenal HCO₃⁻ secretion and the response was inhibited by AE5-599 (EP₃ antagonist).²⁹⁾ The compound also inhibited keratinocyte growth and induced ceramide production.³⁰⁾ SC-46275 was also identified as a selective EP₃ agonist³¹⁾ and it inhibited cytokine release from activated human monocytes.³²⁾ Signaling experiments revealed that MB28767 inhibited forskolin-induced cAMP concentrations in Chinese

hamster ovary cells.³³⁾

iv) EP₄ agonists: ONO-AE1-329 and cicaprost^{16,17)} are well known selective EP₄ agonists (Figures 1F and G). ONO-AE1-329 induced potent vasodilatation of the human pulmonary vein and relaxations induced by PGE₂.³⁴⁾ It also significantly attenuated LPS-induced serum concentrations of TNF-alpha and IL-6 in an endotoxin shock model.³⁵⁾ The PGE₂-induced relaxations were mimicked by an ONO-AE1-329 in aortic rings.³⁶⁾ Recently, we developed a novel EP₄ selective agonist (EP4RAG) (Figure 1H). The EP4RAG was selectively bound to the murine EP₄ receptor.

EFFECTS OF EP₄ AGONISTS ON CARDIOVASCULAR DISEASE

i) Myocardial ischemia: In the development of myocardial infarction and ischemia reperfusion injury, various inflammatory cells migrate into the myocardium and inflammatory cytokines and chemokines are produced.³⁷⁻³⁹⁾ In this situation, PGE₂ shows a cardioprotective effect via attenuation of the activity of inflammatory cells in the heart. Although the exact mechanism of the cardioprotective role of EP₃ is unclear, recent studies have showed that EP₃ is constitutively expressed on cardiomyocytes. It is functionally active and results in an inhibition of adenylate cyclase and subsequent decrease in cardiomyocyte cAMP formation. Thus, EP₃ receptor overexpression on cardiomyocytes reduced ischemic myocardial injury.⁴⁰⁾ Hohlfield, *et al* demonstrated that the properties of EP₃ include inhibition of adenylate cyclase and upregulation during regional myocardial ischemia, suggesting an involvement in the anti-ischemic activity of PGE₂ and EP₃.⁴¹⁾ To analyze the role of EP₄ in the development of myocardial ischemia, we used a novel EP₄ selective agonist called EP4RAG. Of note, EP4RAG significantly reduced the infarction per ischemic myocardium ratio and improved ventricular contraction compared to the vehicle treatment. We also revealed that EP4RAG attenuated chemokine production in the ischemic condition.⁴²⁾

ii) Cardiac transplantation: Cardiac rejection is a major complication in heart transplantation; cytokines and chemokines play critical roles in the development of rejection.⁴³⁾ In the pathophysiology of rejection, expression of PGE₂ and EPs plays a critical role in its progression. Stone, *et al* demonstrated that administration of 16,16-dimethyl prostaglandin E₂ (DMPGE₂), a stable analogue of PGE₂, significantly prolonged the survival of heterotopic rat cardiac allografts.⁴⁴⁾ DMPGE₂ also resulted in long-term graft survival and the development of donor-specific tolerance.^{45,46)} Regarding the above, PGE₂ signal plays a pivotal role in heart transplantation. On the other hand, few papers have examined the effects of each EP in cardiac transplantation. Although one study demonstrated that EP₂ and EP₄ agonists prolonged cardiac allograft survival,⁴⁷⁾ there was little information on the immunological mechanism between EPs and rejection in the report. Therefore, we used EP4RAG in a murine cardiac transplantation model to confirm the role of EP₄ in cardiac rejection.⁴⁸⁾ In that paper, we demonstrated that activation of EP₄ prolonged graft survival and suppressed myocardial cell infiltration. We also showed that EP4RAG inhibited NF-κB activation through the suppression of macrophage activation.

iii) Myocarditis: Myocarditis is an inflammatory heart disease

Figure 2. Summary of the immunological characteristics of EPs.

and cytokines are crucial in its development,^{49,50} thus, PGs and their receptors play a critical role in its pathophysiology.⁵¹ An experimental autoimmune myocarditis (EAM) model has been used to investigate the pathogenesis of myocarditis.^{52,53} We showed that PGE₂ modulated the migratory capacity of different cell types.¹² This contributes to the pathogenesis of inflammatory diseases^{54,55} via EP receptors.⁵⁶ It has been reported that a selective EP4 antagonist completely reversed PGE₂-mediated suppression of chemokine production.¹² Therefore, we investigated the role of EPs in autoimmune myocarditis and clearly demonstrated that EP4RAG significantly suppressed EAM development.⁵⁷

iv) Conclusions: We demonstrated that some EPs mediated the suppressive effects of myocardial inflammatory diseases. In particular, we showed a selective EP4 agonist attenuated inflammatory cytokines and chemokines via attenuation of macrophage activation in cardiac ischemia, transplant rejection, and myocarditis (Figure 2). These findings concerning PGE₂ and EPs will hopefully contribute to the development of new drugs targeting prostanoid-induced inflammatory diseases.

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