

## Review Article

# Activated protein C: a potential cardioprotective factor against ischemic injury during ischemia/reperfusion

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**Abstract:** Activated protein C (APC) is a vitamin-K dependent natural anticoagulant protein. With its function in blood clotting reaction, APC can reduce the risk of venous thrombosis to prevent ischemic disease. A number of *in vivo* and *in vitro* studies over the past few decades have revealed that APC also exerted cytoprotective effects to decrease the mortality caused by endotoxin, sepsis, and brain ischemic stroke. The direct cytoprotective role requires APC binding to the endothelial protein C receptor (EPCR) and activating protease activated receptor-1 (PAR-1). It is now believed that the beneficial characters of APC are partially independent from its anticoagulant activity, though more studies need to be done to demonstrate the exact molecular mechanism. In this review, we have linked the cytoprotective effects of APC including the anti-inflammatory and anti-apoptosis activities to myocardial ischemic injury caused by cardiac ischemia reperfusion. Specifically, we have tried to combine the potential signaling pathways initiated by APC with the well-known adaptive signaling such as AMP-activated protein kinase (AMPK), PI3K/Akt and ERK/MAPK pathways that contribute to the cardioprotection against myocardial ischemia injury. We speculate that APC protects against cardiac ischemia injury via triggering crucial cardioprotective signaling pathways, and these effects are mostly associated with its cytoprotective activity but independent on its anticoagulant activity.

**Key words:** Activated protein C (APC), myocardial ischemia, anti-inflammation, anti-apoptosis, AMP-activated protein kinase (AMPK) pathway, PI3K/Akt pathway, ERK/MAPK pathway

## Introduction

Ischemia heart disease is one of the leading causes of mortality all over the world. There are more than 6 million Americans living with myocardial ischemia and this number keeps increasing all the time [1]. Myocardial ischemia is a disorder of cardiac function that occurs when blood supply to myocardium is insufficient, including low-flow and no-flow ischemia. Patients with ischemia heart disease can often feel chest pain, pressure and discomfort because of the acute reduced oxygen supply to myocardial mitochondria. The decrease blood flow may happen with a number of reasons, such as coronary arteriosclerosis, coronary thrombosis (obstruction by a thrombus) and less frequently, the narrowing of arterioles in the heart. Other risk factors like hyperlipidemia,

hypertension, diabetes as well as insulin resistance, heart failure and aging are also associated with myocardial ischemia [1].

The treatments of myocardial ischemia mainly include rapid recovery of blood and oxygen supply, i.e., reperfusion, which can reduce the mortality by 50%. [1] However, the reperfusion therapy following acute cardiac ischemia can lead to various complications including myocardial infarction, cardiac contractile dysfunction and arrhythmia because of the large reactive oxidative stress generated with the re-admission of oxygen as well as other metabolic products. Therefore, the intracellular mechanism involved in cell necrosis and apoptosis caused by ischemia reperfusion, and potential drugs that help decrease severe cell injury are becoming clinically important.

### Apoptosis, necrosis and ischemia reperfusion injury

Cardiac injury and infarction caused by ischemia reperfusion is through two main mechanisms, apoptosis and necrosis. It is well known that necrosis can occur during ischemic condition, and this was accelerated by reperfusion, with the reintroduction of oxygen [2-4]. However, the controversy is whether the more destruction during reperfusion is due to the irreversible cell necrosis or cell apoptosis caused by ischemia. MuCully has pointed out that during long time global ischemia, necrosis contributes more to the myocardial infarction, whereas apoptosis was inhibited at the very beginning of reperfusion [5]. It was also suggested by Diaz that reperfusion can initiate some salvage signals to act as an acute protection [6]. The proportion of destructive or protective role played by reperfusion needs to be further studied.

The mechanism of ischemic necrosis is mainly caused by ATP depletion during ischemia. Lack of ATP will inhibit Sarcoplasmic Reticulum (SR)  $\text{Ca}^{2+}$  uptake and  $\text{Na}^+/\text{K}^+$  ATPase activity, leading to the increase of intracellular  $\text{Ca}^{2+}$  and  $\text{Na}^+$  concentrations [7, 8]. The accumulation of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  can result in osmotic swelling which can further trigger sarcolemmal rupture. In addition,  $\text{Ca}^{2+}$  overload can initiate protease activation, and the whole irreversible process will cause cell necrosis [9]. The acceleration of myocardial necrosis during reperfusion is largely due to enormous reactive oxygen species (ROS) generated by impaired electron transfer chain on the mitochondrial inner membrane.

Apoptosis caused by ischemia reperfusion injury is initiated via both of mitochondrial and extrinsic death signaling. The raise of ROS during reperfusion causes damage in many functional proteins, resulting  $\text{Ca}^{2+}$  release. The augment intracellular calcium signaling will lead to the formation of mPTP, the multimeric pore structure spanning both outer and inner membrane of mitochondria, and as a result, cytochrome C is released into the cytoplasm to activate caspase family proteins [1]. The extrinsic death signaling requires the some inflammatory factors such as tumor necrosis factor (TNF). It has been reported that TNF- $1\alpha$  level was enhanced in the ischemia reperfusion heart. The inhibition of TNF- $1\alpha$

could dampen cardiac dysfunction after ischemia reperfusion [10].

### Myocardial ischemia reperfusion and inflammatory response

Myocardial ischemia reperfusion injury is associated with acute inflammatory response *in vivo*. One indicator of inflammatory response is the cytokine release. For patients with myocardial infarction, an increased level of pro-inflammatory factors such as interleukin (IL)- $1\beta$ , IL-6, IL-8, TNF- $\alpha$  were detected in their plasma [11, 12], and concentrations of these pro-inflammatory cytokines showed a gender difference, with females having decreased TNF- $\alpha$ , IL-1, and IL-6 levels and less activated p38/MAP kinase signaling compared to age-matched males in rat models[13]. The cytokine production and release can occur in many different cells such as lymphocytes, macrophage, monocytes and tissue endothelial cells [14], which make the mechanism complicated. Besides, the role played by these cytokines in myocardial ischemia reperfusion is more intricate. Sharma indicated in his review that low level of TNF- $\alpha$ , IL-1, and IL-6 can trigger the release of heat shock protein from the endothelium and cardiomyocytes, preventing leukocytes and endothelial cell adhesion and as a consequence, reducing cardiac ischemia reperfusion injury, whereas high cytokine concentration activates leukocytes to produce more free radicals, leading to ischemic injury [15]. However, the clear protective or deleterious functions of these inflammatory factors need to be elucidated.

In addition to cytokine release, inflammatory response also involves other reactions including neutrophile activation, endothelial activation, microphage accumulation and leukocyte adhesion. Activated neutrophile will immigrate to the local inflammatory area and infiltrate into the underlying tissue [16], where it undergoes degranulation to release free radicals [17, 18] and cytotoxic protease [19] that exacerbate myocardial dysfunction and injury. Microphage accumulation in infarct areas can lead to the expression and release of macrophage migration inhibitory factor (MIF). Long time MIF release results in the amplification of cardiac inflammatory and injury [20]. However, recent study pointed out the acute MIF activation in adaption to

ischemic stress activates AMP-activated protein kinase signaling and contributes to the reduced infarct size [21].

### **Cardioprotective signaling during ischemia and reperfusion**

#### *AMPK signaling*

One of the signaling pathways that protect against myocardial ischemia is the AMP-activated protein kinase (AMPK) pathway [22]. AMPK is a stress sensitive kinase that can be activated by ATP depletion such as hypoxia[23], ischemia[24] and exercise [25]. Activated AMPK can phosphorylate Acetyl-CoA carboxylase (ACC) to inhibit its activity engaged in fatty acid synthesis [26]. Other downstream effects of AMPK pathways include glucose uptake [27, 28], glycolysis [29] and fatty acid oxidation [30], which favor the ATP production that supply enough energy for cell survival under the stress conditions.

AMPK is a heterotrimeric protein that consists of  $\alpha$ ,  $\beta$ ,  $\gamma$  subunits. Each of these subunits has at least two isoforms that expressed unequally in different tissues. The  $\alpha$  subunit has two isoforms known as  $\alpha 1$  and  $\alpha 2$ , with  $\alpha 2$  predominant in heart and skeleton muscle [31, 32]. The phosphorylation site of AMPK is Thr 172 on the  $\alpha$  subunit, and mice that lack either  $\alpha 1$  or  $\alpha 2$  can be used as AMPK deficiency model for different purposes. Both  $\beta 1$  and  $\beta 2$  subunit contain glycogen binding site that can exert the regulation of protein activation via intracellular glycogen level [33]. The  $\gamma$  subunit is the key regulatory subunit because it contains AMP binding site. With the binding of AMP, AMPK undergoes conformational change that facilitates the phosphorylation of  $\alpha$  subunit by upstream AMPK kinases. The  $\gamma$  subunit has  $\gamma 1$ ,  $\gamma 2$  and  $\gamma 3$  isoforms.  $\gamma 3$  is absent in heart but predominant in skeleton muscle [34].

Activation of AMPK was identified to be regulated by three distinct upstream kinase cascades. Hawley first reported that LKB1, the tumor suppressor kinase, was associated with AMPK phosphorylation in the liver [35]. Mice with low expression of LKB1 in skeleton muscle failed to activate AMPK or mediate glucose uptake during muscle contraction. Moreover, the expression level of AMPK  $\alpha 2$  subunit is lower than normal mice [36]. Same

phenomenon was observed in LKB1 deficient heart. Both the expression and basal phosphorylation level of AMPK  $\alpha 2$  is less in the heart lacking LKB1, and even ischemia or hypoxia cannot induce AMPK  $\alpha 2$  activation. Instead, the AMPK  $\alpha 1$  phosphorylation is markedly increased in LKB1 deficient heart, suggesting a salvage pathway in cardioprotection [37]. Later in 2005, it was revealed that  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase kinase (CaMKK) serves as a second AMPK upstream kinase [38, 39]. In HeLa cells and murine embryo fibroblasts derived from LKB(-/-) mice, treating with some pharmacological drugs will activate AMPK phosphorylation in CaMKK $\beta$ -dependent manner, indicating the crucial role played by calcium signaling in AMPK activation. [39]. The third AMPK kinase is TAK1, a transforming growth factor-beta-activated kinase that belongs to MAPKKK family. This protein was first identified by applying mouse cDNA library into yeast system that lacked Snf1 (AMPK homologue in yeast) kinase [40]. It has also been found that TAB1, the binding partner of TAK1, can interact with AMPK to mediate p38/MAPK phosphorylation in ischemic heart [41].

The action of AMPK on ischemia reperfusion heart is critical. Russell and colleagues have figured out that mice expressing a kinase dead (KD) form of AMPK failed to increase glucose uptake and fatty acid oxidation during cardiac ischemia reperfusion, which led to the aggravated apoptosis and necrosis in heart tissues [24]. These effects were due to the central role AMPK played in metabolism. One of the main downstream target protein of AMPK is Acetyl-CoA carboxylase (ACC) which converts acetyl-CoA to malonyl-CoA by carboxylation to favor fatty acid synthesis. Phosphorylation of ACC by AMPK inactivates the enzyme [42], and as a result, fatty acid oxidation and ATP production are preferred. Moreover, AMPK can mediate cardiac glucose transporter GLUT4 translocation to the cell surface [27, 28], enhancing glucose uptake during ischemia [24]. On the other hand, chronic activated AMPK is correlated with increase in GLUT4 expression regulated by myocyte-enhancing factor 2A [43]. More interestingly, AMPK contributes to the augment of glycolysis in ischemic heart through stimulating 6-phosphofructo-2-kinase (PFK-2) activity [44]. There is also some cross-

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talk of AMPK cascade with other signaling pathways. AMPK was found to phosphorylate eNOS at Ser1177 site to enhance NO production [45], and NO in return, is associated with promoting glucose uptake regulated by AMPK [27].

### *PI3K/Akt pathway*

PI3K/Akt pathway was originally discovered in insulin signaling. Upon activation by insulin receptor substrate-1 (IRS-1), PI3K will phosphorylate its downstream target Akt/PKB to mediate glucose uptake in response to high insulin level. Furthermore, PI3K can be activated via the interaction with  $\beta\gamma$  heterodimer of G protein in G-protein coupled receptor signaling [46], as well as in receptor tyrosine kinase signaling. Therefore, the phosphorylation of Akt can be stimulated by many growth factors, such as insulin growth factor (IGF-1) [47], endothelial growth factor (EGF) [48] and transforming growth factor (TGF- $\beta$ ) [49].

It has been reported that ischemia preconditioning can increase the release of some cardioprotective ligands such as adenosine [50], which will trigger the receptor tyrosine kinase activity via G-coupled protein receptor. Various downstream protein kinases are activated, including PI3K/Akt cascade [51]. Hilary stated that by using mice expressing heterozygous PTEN, a phosphatase in PI3K/Akt pathway, in globe ischemia reperfusion model with ischemic preconditioning, the infarct size was reduced compared to littermate. In addition, the phosphorylation of Akt was increased [52]. Also, activation of Akt has been found in single cardiomyocytes with simulated ischemia reperfusion, but not with ischemia alone, and treating with tyrosine kinase inhibitor or p38 MAPK inhibitor can at least partially impede Akt phosphorylation, suggesting the important role of tyrosine kinase in Akt activation during ischemia reperfusion [53].

The downstream targets of PI3K/Akt include Bcl-2 family members Bax/Bad, endothelial nitric oxide synthase (eNOS), P70 S6 kinase (P70S6P) and glycogen synthase-3 $\beta$  (GSK-3 $\beta$ ). The mitochondrial translocation of Bax stimulated by ischemia was hindered with the reduced 14-3-3 phosphorylation level mediated via Akt activation, which acts as anti-

apoptosis pathway against ischemic injury [54]. Phosphorylation of GSK-3 $\beta$  by Akt inactivates the enzyme, leading to the reduced infarct size and the improved recovery of left ventricle diastolic pressure (LVDP) after ischemia preconditioning, and using GSK-3 $\beta$  inhibitor can mimic the cardioprotective effect of phosphorylated GSK-3 $\beta$  [55]. Moreover, Akt can phosphorylate eNOS to increase NO production. NO released from vascular endothelial cells can help relax and dilate the blood vessel, and help prevent leukocyte adhesion, decreasing atherosclerosis. More importantly, NO can enter cells and target guanylate cyclase to generate cGMP, which result in the activation of PKG. One of the downstream effects by PKG is to lower cytosolic calcium overload by inhibiting intracellular calcium release from ER and extracellular calcium uptake [56]. Also, PKG can directly regulate eNOS to control the cGMP production, which is a feedback effect [57].

### *ERK/MAPK pathway*

Among the three mitogen-activated protein kinase (MAPK) modules, ERK/MAPK activation can regulate cell proliferation and differentiation, inhibiting apoptosis. Like PI3K/Akt pathway, the phosphorylation of ERK/MAPK is identified during ischemia reperfusion, and this activation of ERK is thought to be one of the major protective signaling pathways involved in both ischemic preconditioning and postconditioning [1].

Activation of ERK/MAPK is also dependent on G protein-coupled receptor (GPCR) [58]. Agonists such as adenosine, bradykinin and opioids released during acute ischemia reperfusion can bind to specific receptors to trigger GPCR signaling, including PI3K and ERK pathways [59]. Fryer has reported the cardioprotective effects (reduced infarct size) of opioids was largely abolished by using PD 098059, a pharmacological inhibitor of ERK upstream kinase MEK, indicating the important role played by ERK/MAPK in myocardial adaptation to ischemic stress [60]. Besides, ERK phosphorylation can be mediated by protein kinase C (PKC). Overexpression of PKC $\epsilon$  in cardiomyocytes stimulates phosphorylation of ERK1/2, leading to the reduced level of lactate dehydrogenase release induced by hypoxia [61].

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Unlike other MAP kinases, ERK acts as anti-apoptosis signaling [62]. Activated ERK1/2 can directly or indirectly inhibit caspase activation, even in the presence of death signaling from Fas receptor [63]. Moreover, ERK signaling can induce Bcl-2 and Bcl-X(L) expression [64] and regulate phosphorylation of Bcl-2 [65]. Treated with ERK specific inhibitor, PD98059, the perfused rat heart showed impaired recovery after global ischemia and increased apoptosis in cardiomyocytes [66].

### Activated protein C

Activated protein C (APC) is a vitamin-K dependent serine protease that inhibits blood clotting in plasma [67]. It is activated from the circulating protein C zymogen on the surface of endothelial cells. Sequence analysis revealed that protein C is consisted of multiple domains. The catalytic domain contains trypsin-like serine protease activity [68], and the Gla-domain has nine  $\gamma$ -carboxyl glutamic acid residues [69]. Two epidermal growth factor (EGF) - like domains serves as the linking peptides [70]. The anticoagulant function of APC involves the interaction of the protease Gla-domain with the cofactor protein S to degrade factor V and factor VIII in coagulant cascade [71]. Besides, APC has other effects including anti-inflammatory, anti-apoptosis effects and mediating gene expression. Its benefits in cytoprotection cannot be explained only by the anti-coagulant activity because other anticoagulants, e.g. heparin, do not have similar protective functions [72]. Therefore, to address the molecular and cellular mechanisms of APC action on different diseases such as sepsis, stroke and ischemia is of both basic scientific and clinical interests.

### Anticoagulant activity of activated protein C

Protein C normally circulates in the blood at the concentration of 65 nM [73]. Activation of protein C is initiated by the production of thrombin [74]. When blood clotting occurs, prothrombin is cleaved by factor X and factor V to form thrombin. Once interacting to its endothelial receptor thrombomodulin, thrombin can direct the activation of endothelial protein C receptor (EPCR) bound protein C zymogen [75]. The produce rate of APC largely depends on the concentration of

EPCR which varies in different types of cells [76]. After activation, APC will release from EPCR and circulates in the blood, where it interacts with its cofactor protein S. The APC-protein S complex can degrade factor Va and factor VIIIa on the surface of platelets or other cell types, and this anti-clotting complex is reported to be associated with phospholipids [77]. Mutations in factor V (e.g. Arg 506-Glu506) will cause it resistance to the cleavage by APC [78], and people who are APC resistance have a high risk of thrombosis development.

### Anti-inflammatory activity of activated protein C

Different from anti-coagulant activity, the Anti-inflammatory activity of APC is EPCR-dependent. This cytoprotective effect requires the participation of another co-receptor, protease-activator receptor-1 (PAR-1) [79]. PAR-1 is a G-protein coupled receptor that can be mediated by both APC signaling and thrombin signaling, but PAR-1 cleavage and its downstream effects are dissimilar [80]. Beside the expression in endothelial cells, PAR-1 was detected in various cell types, including cardiac fibroblasts and cardiomyocytes [81]. However, EPCR is primarily located on endothelium cells of arteries, vein and capillary [82]. Recently research indicates that EPCR is also expressed on the membrane of neutrophils [83], leukocytes, monocytes [84] and hematopoietic stem cells [85], but there is no report about its expression on cardiomyocytes. Since EPCR-APC complex contributes, at least in part, to the cleavage and activation of PAR-1 as well as its downstream effect, the intracellular signaling of APC is preferential on cells with EPCR expression. Nevertheless, opinions rise recently about the PAR-1 activation via APC in EPCR-independent fashion [72]. O'Brien has pointed out in his study that in HUVECs treated with EPCR siRNA or EPCR antibody, APC can still stimulate phosphorylation of ERK1/2 and upregulate transcription factor EGR-1 activity to decrease TRAIL level, indicating APC activating PAR-1 could be EPCR independent [86]. However, whether siRNA or antibody can completely leave out EPCR in APC-PAR-1 signaling remains questionable. Moreover, other evidence seems to support this EPCR-independent idea. In purified system, PAR-1 can be directly activated with high dose of APC

mutant that lacks EPCR binding domain in cultured cells [80], suggesting the role of EPCR to facilitate PAR-1 cleavage by APC at low concentration. In this case, the activation of PAR-1 in normal physiological condition still requires EPCR participation.

APC acts against inflammation via the inhibition of inflammatory gene expression, inhibition of cytokine release and attenuation of adhesion molecules release. However, the molecular mechanism of how APC functions in these physiological conditions is still not well identified. APC can downregulate proinflammatory cytokines release from both endothelial cells and leukocytes. These inflammatory cytokines include interleukin-6 (IL-6), IL-8 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [87]. In EPCR deficient mice, continuous peritoneal infusion of lipopolysaccharide (LPS) can increase the heart rate and central blood flow pressure. Meanwhile, the expression level of chemokine, microphage inflammatory chemokine (MIP-2) was increased and there were more damage in heart muscle of EPCR deficiency, suggesting the cardioprotective role of APC-EPCR signaling in LPS-induced endotoxemia mice. Interesting, although the mRNA level of IL-6, IL-8 and TNF- $\alpha$  were upregulated in EPCR deficient mice compared to their wild-type littermate, the responses appeared to be the same among the two genotypes. However, the expression level of HIF1 $\alpha$  was significantly higher in deficient mice [88]. Using monoclonal antibody to block APC-EPCR binding in *Escherichia coli* infected mice can lead to an intense flow of neutrophils in different tissues such as renal, adrenal etc. Also an increased plasma level of IL-6 and IL-8 was found in mice receiving *E. coli* together with the blocking antibody of EPCR, indicating that APC-EPCR signaling contributes to the host defense against *E. coli*-induced sepsis via the anti-inflammatory activity [89]. The expression of EPCR was also characterized in monocytes [90] and administration of APC (50 $\mu$ g/ml) markedly inhibited macrophage migration inhibitory factor (MIF) release induced by LPS [91]. The regulation of gene expression by APC is reviewed to be associated with the inhibition of nuclear factor- $\kappa$  B (NF- $\kappa$ B), one of the transcription factors that mediate pro-inflammatory gene expression [92]. However, the mechanism of how APC suppresses NF- $\kappa$ B activity is poorly understood.

### Anti-apoptosis activity of activated protein C

The anti-apoptosis effect of activated protein C also requires its interaction with the cell membrane receptor EPCR. Activation of PAR-1 by APC (mostly in EPCR dependent manner) triggers G-protein signaling, including alteration of cytosolic calcium flux. Treated with human recombinant APC, human brain endothelial cells (BECs) has an increased level of intracellular calcium concentration, and Domotor showed that the calcium resource is from ER release [93]. The alteration in calcium signaling could be one explanation of ERK1/2 phosphorylation by APC [79]. The downstream effects of activated ERK1/2 contain the restraint of mitochondrial permeability transition pore (mPTP) opening in response to extracellular stress such as ischemia. As a result, less cytochrome C was released into the cytoplasm and apoptosis signaling was prevented [94].

Furthermore, APC reduces apoptosis via the inhibition of p53, the transcription factor that regulates pro-apoptosis gene expression [95]. TUNEL positive endothelial cells induced by hypoxia were reduced with APC treatment and lactate dehydrogenase release was also decreased. These effects are EPCR and PAR-1 dependent because the pretreatment of either EPCR or PAR-1 antibody could not diminish the apoptotic cells. Moreover, downregulation of Bax protein and upregulation of Bcl-2 protein were observed in APC treated hypoxic endothelial cells, suggesting that APC can blunt intrinsic apoptotic pathway.

On the other hand, APC signaling counteracts extrinsic apoptotic pathways through the block of vascular and neuronal toxicities potentiated by tissue plasminogen activator (tPA) [96]. In tPA-triggered apoptotic brain endothelial cells, APC exerts pro-apoptotic activity via the inhibition of caspase-8 and caspase-3 activation, leading to the restraint of caspase-3-dependent nuclear translocation of apoptosis-inducing factor.

The anti-apoptotic activities of APC against both intrinsic and extrinsic apoptotic signaling were illustrated in human brain endothelial cells, and APC was thought to be neuroprotective [97]. Further work is needed to identify its anti-apoptotic activity in other tissues such as cardiomyocytes. In addition,

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the mechanism of how APC exerts the inhibition on pro-apoptotic gene expression and whether APC has other effects against apoptosis remains to be clarified.

### Activated protein C in ischemic injury

Based on animal experiment and clinical observations, it has been viewed that the plasma protein C might be protective for ischemic stroke. Also, the prospective epidemiologic Atherosclerosis Risk in Communities (ARIC) study reported that APC is a potential therapy for ischemic injury [97]. A bunch of *in vitro* studies revealed that APC protects against cell injury and apoptosis triggered by hypoxia [95].

The *in vivo* protective effect of APC is relatively well established in neural system. The ischemic infarction of the brain is more severe in EPCR deficient mice compared to the normal littermate control, even in the treatment of APC. Wild-type C57BL/6 mice have bigger ischemic infarct size in the brain with the treatment of PAR-1 antibody. Additionally, in mice subjected to focal ischemia, the motor neurological score and the total brain injury volume, as well as fibrin deposition were significantly lower with the administration of APC (0.2mg/kg) [95]. Other studies reported that in a murine model of focal ischemia, APC administration (2mg/kg) before or after reperfusion can reduce brain infarct volume and brain edema. Moreover, neutrophil infiltrations, as well as the number of fibrin-positive vessels were observed to be less in APC treated mice, suggesting that APC is neuroprotective in ischemic injury [98].

Besides its neuroprotective function, APC has been reported to reduce ischemia reperfusion induced injury in other tissues. In rat model of stimulated spinal cord and renal ischemia, less damage was observed in APC administration group, and APC attenuates the increase of TNF- $\alpha$ , IL-8 and myeloperoxidase among these tissues triggered by ischemic stress. It has also been pointed out that the protective effect of APC is independent on its anti-coagulant activity because neither inhibitor of thrombin (DEGR-treated factor Va) nor heparin can produce such effects [99, 100].

Early review established the linkage between activated protein C resistance and deficiency

with myocardial infarction in the perspective of preventing coronary occlusion [101]. Clinical studies demonstrated that people with the presence of factor V Leiden, a mutation on factor V causing resistance to activated protein C, have nine fold increase risk of developing ischemic heart disease before the age of 45 [102]. Additionally, mutation in thrombomodulin is associated with myocardial infarction in ways that impairs the generation APC under the normal physiological conditions [103, 104]. By studying the endothelial protein C receptor polymorphisms, Medina reported that haplotypes A1 and A3 in EPCR lead to a reduced risk of premature myocardial infarction due to the increase of APC level in plasma [105].

Aspects of APC activity after myocardial ischemia reperfusion have been studied in both porcine and murine model. Treating APC during ischemia reperfusion process helps reduce the mortality rate and apoptotic rate, and restore the mean arterial pressure without any effect on heart rate or left ventricle pressure after short time occlusion [106, 107]. However, the mechanism behind these physiological improvements stimulated by APC is still unclear.

Further work is needed to illustrate the specific intracellular signaling initiated by APC-EPCR complex, and how it contributes to the protection against ischemic injury in the heart. Moreover, different APC mutants can be generated to test whether the cardioprotection of APC is associated with its anticoagulant or cytoprotective effects, or both.

### Conclusion

Myocardial ischemic disease is one of the leading threats to human health and ischemia reperfusion will lead to local cell injury and systemic inflammation in the body. Activated protein C is a natural anti-coagulant protein that helps prevent coronary occlusion by thrombolysis. Moreover, the cytoprotective effects of APC include reducing the inflammatory response and mediating cell survival by inhibiting cell apoptosis. In a word, APC has the potential protective effects against ischemia reperfusion injury in the heart and the molecular mechanism stimulated by APC remains further investigation.

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