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## *Review*

# Anti-apoptotic effects and mechanisms of salvianolic acid A on cardiomyocytes in ischemia-reperfusion injury

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**Summary.** Prompt myocardial reperfusion during acute myocardial infarction by fibrinolytic therapy, percutaneous coronary intervention, or coronary artery bypass grafting limits the affected area and improves prognosis. However, reperfusion itself can cause cardiomyocyte damage and decrease treatment efficacy. No treatments that effectively prevent myocardial ischemia/reperfusion (I/R) injury are currently available, and are therefore the focus of ongoing research. Salvianolic acid A (SAA), the active ingredient of the traditional Chinese herbal remedy *Salvia miltiorrhiza*, has anti-thrombotic activity, anti-inflammatory, and anti-cancer activity; regulates blood lipids and provides hepatic and neural protection. Recent studies demonstrated that SAA inhibits cardiomyocyte apoptosis in response to I/R by the PI3K/Akt, GSK-3β, JNK, and ERK1/2 pathways, and by JNK-ERK1/2 crosstalk. The mechanisms for SAA attenuating cardiomyocytes apoptosis during I/R injury through the P38 MAPK, caspase, JAK/STAT, NF-κB and LOX-1 signaling pathways need further illustration. There may be potential crosstalks between PI3K/Akt and JNK, and Akt/GSK-3β and ERK1/2 in the process of SAA against I/R-incuced cardiomyocytes apoptosis. This review summarizes the recent evidence of the anti-apoptotic effects and mechanisms of SAA against myocardial I/R injury and discusses the basis of potential clinical applications of SAA.

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**Key words:** Salvianolic acid A (SAA), Ischemia/reperfusion (I/R), Cardiomyocyte, Mechanism, Apoptosis

## Introduction

Acute myocardial infarction (AMI) following thrombotic occlusion is the leading cause of death worldwide, with a high global prevalence (1.92, 2.21, and 4.13 cases per 1000 person-years in high-, middle-, and low-income countries) (Anderson and Morrow, 2017). The cascade of thrombotic events after the abrupt rupture of an atherosclerotic plaque results in acute occlusion of the coronary artery, which blocks the supply of blood and oxygen to cardiomyocytes; ultimately leading to AMI (Anderson and Morrow, 2017). Persisting occlusion of the myocardial blood supply interrupts the washout of catabolic products in the region of the infarct, which results in myocardial structural damage and decline in function. The severity of ischemia and timing of the ischemic event and reperfusion strategy influence the infarct size, which is the major determinant of prognosis (Reimer and Jennings, 1979; McKay et al., 1986). Reperfusion therapy to reopen the occluded coronary artery and promptly restore blood flow to the ischemic cardiomyocytes reduces the duration and degree of ischemia. Reperfusion can be achieved by fibrinolytic treatment, percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG) (Anderson and Morrow, 2017). Timely and effective reperfusion is essential to minimize the size of an AMI and to preserve

cardiomyocyte contractility, and is the only way to salvage ischemic cardiomyocytes (Estevez-Loureiro et al., 2014). Paradoxically, reperfusion triggers an oxidative burst, calcium overload, and mitochondrial damage that collectively induce cardiomyocyte apoptosis and necrosis, resulting in irreversible damage described as myocardial ischemia-reperfusion (I/R) injury (Braunwald and Kloner, 1985; Yellon and Hausenloy, 2007).

The cardiac pathophysiology of I/R includes four changes, reperfusion arrhythmia, myocardial stunning, microvascular obstruction, and lethal reperfusion injury (Yellon and Hausenloy, 2007; Hausenloy and Yellon, 2013; Altamirano et al., 2015). These changes may reduce the benefits of myocardial reperfusion, and the severity of I/R injury strongly affects the treatment effectiveness and prognosis in patients with AMI (Jennings, 2013; Zhu et al., 2017). Myocardial I/R injury is a complex pathological process that involves oxidative stress, calcium overload, mitochondrial damage, and apoptosis (Yellon and Hausenloy, 2007; Hausenloy and Yellon, 2013; Altamirano et al., 2015); it is the primary cause of heart failure after AMI (Heusch et al., 2010). Myocardial I/R injury was found to contribute to as much as half the final AMI size in animal models (Yellon and Hausenloy, 2007). Unfortunately, there is still a lack of effective clinical treatments for myocardial I/R injury (Hausenloy and Yellon, 2013; Oerlemans et al., 2013). Finding drugs to improve heart function, overcome the detrimental effects, reduce the morbidity and mortality of myocardial I/R, and limit the size of AMI are priorities.

The use of herbal remedies to prevent and treat myocardial I/R injury has increased with the prevalence of AMI. The dried roots and rhizomes of *Salvia miltiorrhiza* are included in a traditional Chinese herbal remedy used for the treatment of cardiovascular diseases by promoting blood circulation and by resolving blood stasis (Lin et al., 2006; Yue et al., 2012). The active ingredients are hydrophilic depsides and lipophilic diterpenoid quinones (Li et al., 2009). Early studies focused on lipophilic diterpenoid quinones while recent studies focused on hydrophilic depsides.

Salvianolic acid A (SAA, Fig. 1), the main hydrophilic depside of *Salvia miltiorrhiza*, possesses multiple pharmacological activities, including anti-thrombotic (Yuan et al., 2017), anti-inflammatory (Li et al., 2016; Yuan et al., 2017), blood-lipid regulatory (Ding et al., 2016), hepatoprotective (Ding et al., 2016), neuroprotective (Feng et al., 2017) and anti-cancer (Tang et al., 2017). Recent studies found that apoptosis is a key event in myocardial I/R injury and the cardioprotective activity of SAA during myocardial I/R depended on inhibition of apoptosis *in vivo* and *in vitro*, but the ways in which the apoptosis pathways were affected are not known (Pan et al., 2011; Fan et al., 2012; Xu et al., 2014; Li et al., 2017). This review describes the signaling pathways that regulate cardiomyocyte apoptosis and the protective effects against cardiac I/R injury that derive from the anti-apoptotic activity of SAA. The aim is to reveal potential therapeutic applications of SAA as a modulator of pro- and anti-apoptotic pathways in cardiomyocytes. Cardiomyocyte protection could reduce AMI size and improve clinical outcomes of myocardial I/R injury.

## Mechanisms of cardiomyocyte apoptosis during I/R

Cardiomyocyte apoptosis, a key event in the occurrence and progression of cardiovascular disease, is an active, orderly form of myocardial cell death following a variety of pathological events (Chang et al., 2011). During I/R, reperfusion of the region affected by the AMI following anti-thrombotic and mechanical treatment triggers a cascade of pathophysiological events that result in cardiomyocyte apoptosis. Reperfusion may be a stronger stimulus for cardiomyocyte apoptosis than persistent ischemia (Gottlieb et al., 1994; Fliss and Gattinger, 1996). Activation of apoptosis pathways in cardiomyocytes occurs during reperfusion following the translation of the early signaling events that occur during ischemia (Yaoita et al., 2000; Oerlemans et al., 2013). Apoptosis is known to be responsible for myocardial I/R injury, and the extent of cardiomyocyte apoptosis is a key determinant of the severity of the injury (Fliss and Gattinger, 1996; Fan et al., 2012; Liu et al., 2017). The molecular basis of cardiomyocyte apoptosis has not been completely described, but oxidative stress, calcium overload, and mitochondrial damage have been implicated.

During I/R, cardiomyocytes that were stressed by the AMI-induced insufficiency of blood and oxygen are subject to the formation of myocardial reactive oxygen species (ROS), superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide ($H_2O_2$) (Feuerstein and Young, 2000). The restoration of blood flow after prolonged ischemia is accompanied by accumulation of large quantities of ROS that exceed the antioxidant capacity of cardiomyocytes. The resulting lipid peroxidation and calcium overload open the mitochondrial membrane permeability transition pores

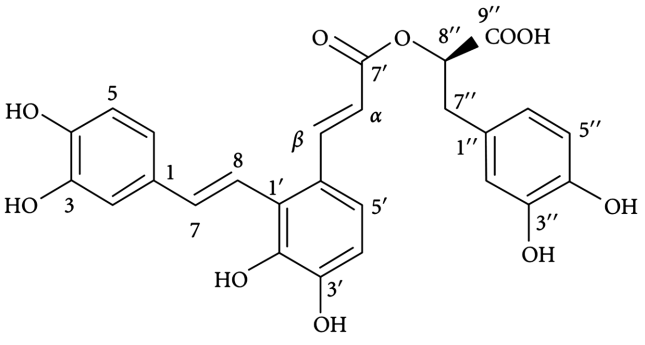


**Fig. 1.** Chemical structure of salvianolic acid A (SAA).

(mPTPs) and ultimately trigger cardiomyocyte apoptosis (Hausenloy and Yellon, 2013; Zhang et al., 2017b). In addition to being the most important place for the generation of ROS by activation of the electron transport chain, the mitochondria are particularly sensitive to oxidative stress. In response to the oxidative stress during I/R, mitochondria undergo pathophysiological changes that include damage of the mitochondrial membrane by lipid peroxidation and mPTP opening, mitochondrial swelling, metabolic disorders, and the activation of caspase cascades (Heusch et al., 2010; Li et al., 2010). Calcium accumulation triggers the opening of mPTPs that leads to collapse of the mitochondrial membrane potential and ionic homeostasis, which further increases calcium overload during I/R. This complex network of interactions increases mitochondrial membrane permeability, promotes cytochrome c (Cytc) release, and activates caspase cascades that induce apoptosis. Sustained mPTP opening leads directly to cardiomyocyte injury, while transient mPTP opening leads to cardiomyocyte apoptosis (Zamzami et al., 2005); mPTPs are thus potential targets of anti-apoptotic treatment during myocardial I/R injury.

## SAA and the signaling pathways affecting cardiomyocyte apoptosis following I/R

Signaling pathways that have been reported to mediate cardiomyocytes apoptosis during I/R injury include the phosphatidylinositol-3-kinase/Akt (PI3K/Akt), glycogen synthase kinase 3β (GSK-3β), mitogen-activated protein kinases (MAPKs), janus kinase/signal transducer and activator of transcription (JAK/STAT), caspase, nuclear factor- κB (NF-κB), and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1). PI3K/Akt, MAPKs, and JAK/STAT are classical survival signaling pathways; GSK-3β, NF-κB and LOX-1 are pro-apoptotic pathways; and caspase signaling can either promote or inhibit apoptosis. SAA can activate the PI3K/Akt/GSK-3β pathway to upregulate Bcl-2 expression, which suppresses the expression of caspase-3 and inhibits apoptosis (Pan et al., 2011; Fan et al., 2012; Li et al., 2017). SAA also has anti-apoptotic effects during myocardial I/R injury via the MAPKs (Fan et al., 2012; Xu et al., 2014; Yang et al., 2014; Zhang et al., 2017a), JAK/STAT, NF-κB, and LOX-1 signaling pathways (Fig. 2).

### *SAA, the PI3K/Akt pathway, and cardiomyocyte apoptosis following I/R*

The PI3K/Akt pathway is an anti-apoptotic kinase signaling pathway that promotes cell survival, and regulates growth, differentiation, apoptosis, and autophagy (Duan et al., 2015; Jiang et al., 2016). Activation of the PI3K/Akt pathway is known to protect cardiomyocytes from I/R injury by inhibiting apoptosis (Duan et al., 2015; Nai et al., 2015). Following PI3K activation, phosphatidylinositol (4,5)-bisphosphate is converted to phosphatidylinositol (3,4,5)-triphosphate, and Akt is activated in the plasma membrane. Akt is a downstream target of PI3K, and activated Akt phosphorylates multiple substrates that suppress the expression of Bad and Bax pro-apoptotic proteins and

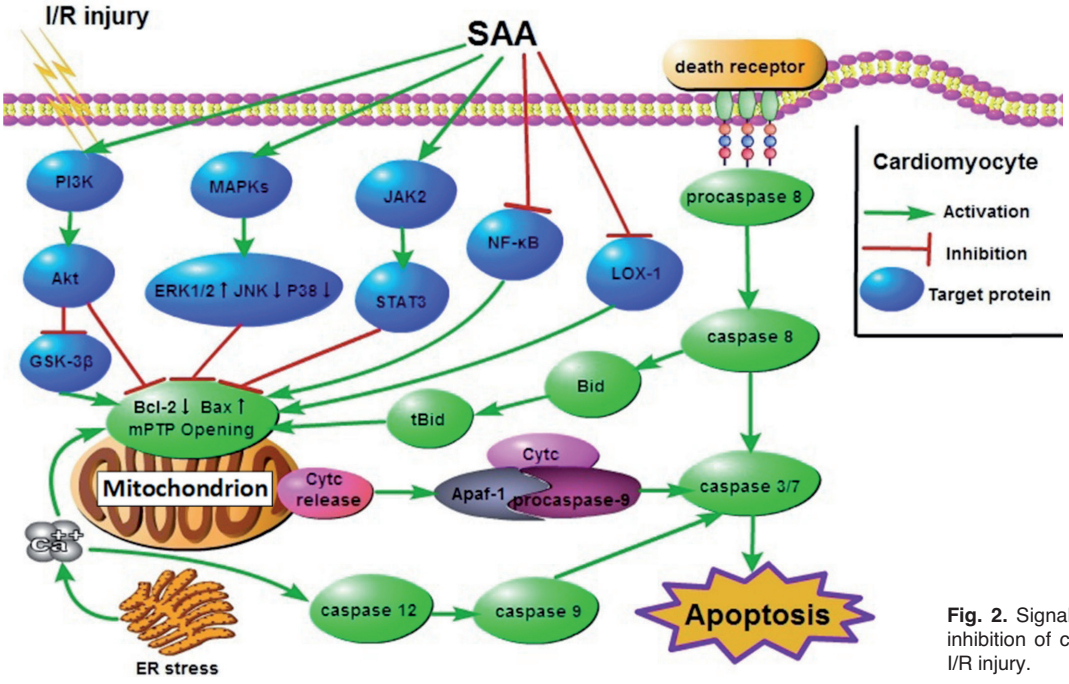


**Fig. 2.** Signaling pathways involved in SAA inhibition of cardiomyocyte apoptosis during I/R injury.

promote the expression of the anti-apoptotic protein Bcl-2 (Cantley, 2002). Cardiomyocyte survival and apoptosis depend on the balance of pro- and anti-apoptotic proteins (Cory and Adams, 2002), and the Bcl-2/Bax ratio may be a reliable marker of cardiomyocyte apoptosis (Korsmeyer et al., 1993).

Experimental evidence supports a role of SAA in inhibiting apoptosis associated with myocardial I/R injury through activating the PI3K/Akt pathway, which increases Bcl-2 expression and the ratio of Bcl-2/Bax *in vivo* (Fan et al., 2012) and *in vitro* (Pan et al., 2011; Fan et al., 2012; Li et al., 2017). These study results indicated that SAA limited infarct size and increased cell viability by reducing cardiomyocyte apoptosis by activating the PI3K/Akt pathway and upregulating Bcl-2 expression. A few studies have investigated the effect of SAA pretreatment on shortening of cardiomyocyte contraction amplitude during I/R injury (Pan et al., 2011), which is a sensitive marker of heart contractile function (Hao et al., 2009; Xu et al., 2010). SAA pretreatment markedly improved cardiomyocyte shortening amplitude and contractile function in isolated rat hearts by reducing I/R-associated necrosis and apoptosis. SAA pretreatment also increased Akt and Bcl-2 expression and decreased Bax and caspase-3 expression. The upregulation of Akt expression was partially attenuated by the PI3K inhibitor, LY294002. The experimental evidence is consistent with SAA-mediated inhibition of I/R-induced cardiomyocyte apoptosis by the PI3K/Akt pathway and its dependence on the balance of Bcl-2 and Bax expression.

### *SAA, Glycogen synthase kinase 3β and cardiomyocyte apoptosis following I/R*

GSK-3β, a downstream Akt target, promotes cardiomyocyte apoptosis after AMI (Woulfe et al., 2010). Phosphorylation of GSK-3β can be mediated by Akt; signaling pathways that activate Akt can inhibit GSK3-3β by phosphorylating inhibitory serine sites (Litwiniuk et al., 2016). Li et al. (2017) found that SAA pretreatment significantly inhibited cardiomyocyte apoptosis after myocardial I/R by increasing the expression of p-Akt, p-GSK-3β and Bcl-2/Bax and by decreasing the opening of mPTPs and caspase-3 expression in primary cultures of neonatal rat cardiomyocytes. The PI3K inhibitor, LY294002, was used to determine whether PI3K/Akt pathway activity was associated with increased p-GSK-3β expression. LY294002 significantly inhibited the increase of p-GSK-3β expression that was mediated by SAA, which was consistent with inhibition of the anti-apoptotic effect of SAA via the PI3K/Akt/GSK-3β pathway.

### *SAA, the MAPKs pathway, and cardiomyocyte apoptosis following I/R*

MAPKs are evolutionarily conserved serine/threonine kinases that are active in cell proliferation and survival. Three members of the MAPK family mediate cardiomyocyte apoptosis; extracellular signal-regulated kinases (ERK1/2), C-jun N-terminal kinase (JNK), and P38 MAPK (Rose et al., 2010). Activation of ERK1/2 has been associated with the inhibition of cardiomyocyte apoptosis after I/R (Fan et al., 2012; Wu et al., 2013; Xu et al., 2014) while JNK activation had the opposite effect (Ferrandi et al., 2004; Xu et al., 2014); the role of P38 MAPK remains controversial (Rose et al., 2010).

Inhibition of apoptosis by SAA by activation of ERK1/2 and inhibition of the JNK pathway has been shown in several studies (Fan et al., 2012; Xu et al., 2014). Fan et al. (2012) reported that SAA pretreatment increased the phosphorylation of ERK1/2 and upregulated Bcl-2 expression in I/R cardiomyocytes *in vivo* in a rat heart I/R injury model and *in vitro* in $H_2O_2$-induced H9c2 cardiomyocytes. Inhibition of I/R-induced cardiomyocyte apoptosis by SAA was achieved by the activation of the ERK1/2 pathway and increase of Bcl-2 expression. In a previous study, we found that SAA pretreatment during myocardial I/R injury caused an increase in ERK1/2 and Bcl-2 expression and a concurrent decrease in JNK, Bax, and caspase-3 expression (Xu et al., 2014). SAA thus prevented cardiomyocyte apoptosis by modulating the ERK1/2 and JNK pathways.

Effects of the ERK1/2, JNK and p38 MAPK pathways on cardiomyocyte apoptosis may be contradictory. Yang et al. (2014) reported that the activation of the P38 MAPK pathway induced by I/R in cultured human umbilical vein endothelial cells was abolished by SAA pretreatment. Zhang et al. (2017a) found that SAA prevented arsenic trioxide-induced apoptosis in H9c2 cardiomyocytes in part by inhibiting P38 MAPK expression. The cardioprotective effect of P38 inhibition was associated with the upregulation of Bcl-2 expression (Kaiser et al., 2004; Ren et al., 2005). However, there is no direct evidence that the P38 MAPK pathway contributes to the inhibitory effects of SAA against I/R-induced cardiomyocytes apoptosis.

### *SAA, the caspase pathway, and cardiomyocyte apoptosis following I/R*

Caspases are key regulators of cardiomyocyte apoptosis during myocardial I/R injury and heart failure (Crow et al., 2004; Regula and Kirshenbaum, 2005). Caspase-mediated apoptosis includes the mitochondria-mediated intrinsic pathway, death receptor-mediated extrinsic pathway, and the endoplasmic reticulum (ER) stress-mediated pathways (Ferri and Kroemer, 2001; Maenpaa et al., 2008), which may have convergent execution. Mitochondrial apoptosis can be induced by oxidative stress, calcium overload, and DNA damage, which results in Bax/Bak-dependent mPTP opening and Cytc release from mitochondria into the cytosol. Cytc binds to apoptotic protease activating factor-1 (Apaf-1), activating coupling with procaspase-9 to form apoptosomes and activate caspase-3/7, which ultimately

results in cardiomyocyte apoptosis (Boatright and Salvesen, 2003).

The death receptor pathway is triggered by extracellular death ligand-binding of tumor necrosis factor (TNF)-α and Fas to their respective death receptors (Peter and Krammer, 2003). Subsequent activation of caspase-8 and caspases 3/7 (Oerlemans et al., 2013) trigger apoptosis (Cohen, 1997; Thornberry and Lazebnik, 1998). ER stress impairs protein folding, calcium homeostasis, and lipid biosynthesis and activates calcium-dependent mitochondrial apoptosis, in which the Bcl-2 family is involved (Sano and Reed, 2013; Urra et al., 2013).

SAA pretreatment has been shown to inhibit cardiomyocyte apoptosis by upregulating Bcl-2 expression and downregulating Bax and caspase-3 expression, which is consistent with the involvement of mitochondrial apoptosis (Pan et al., 2011; Fan et al., 2012; Xu et al., 2014; Li et al., 2017). Li et al. (2017) found that SAA pretreatment significantly suppressed mitochondrial apoptosis in neonatal rat cardiomyocytes in an *in vitro* in I/R model by preserving the mitochondrial membrane potential (ΔΨm) and Bcl-2/Bax and inhibiting the opening of mPTPs and caspase-3 expression. The experimental evidence thus indicates that SAA prevents cardiomyocyte apoptosis from I/R injury via the mitochondrial apoptosis pathway.

Xie et al. (2015) reported that SAA pretreatment alleviated hypoxia-induced endothelial apoptosis by inhibiting ER stress. Yan et al. (2015) found that decreases in ER stress-related proteins were associated with SAA-mediated inhibition of TNF-α/D -GalN-induced apoptosis in hepatocyte LO2 cells. Whether SAA inhibits ER stress-mediated apoptosis in cardiomyocytes during I/R through pathway has not been investigated.

### *SAA, the JAK/STAT pathway, and cardiomyocyte apoptosis following I/R*

The JAK/STAT pathway is a highly conserved modulator of I/R injury. The balance of pro- and anti-apoptotic activity is determined by a network of signaling pathways that interact with the JAK/STAT pathway (Wagner and Siddiqui, 2009). Previous studies found that activated STAT3 triggered anti-apoptotic effects, and that STAT1 had pro-apoptotic activity (Boengler et al., 2008; Wagner and Siddiqui, 2009). Recent evidence indicates that JAK2/STAT3 activation has anti-apoptotic effects in cardiomyocytes in I/R injury models. The effects were associated with the mitochondrial pathway and were abolished by the JAK2-specific inhibitor, AG490 (Yang et al., 2013; Jiang et al., 2015). Whether SAA can inhibit cardiomyocyte apoptosis associated with the JAK2/STAT3 pathway is not known.

### *SAA, the NF- κB pathway, and cardiomyocyte apoptosis following I/R*

NF-κB can induce inflammatory factors that contribute to cardiomyocyte apoptosis. Inhibition of NF-κB was shown to inhibit apoptosis in H9c2

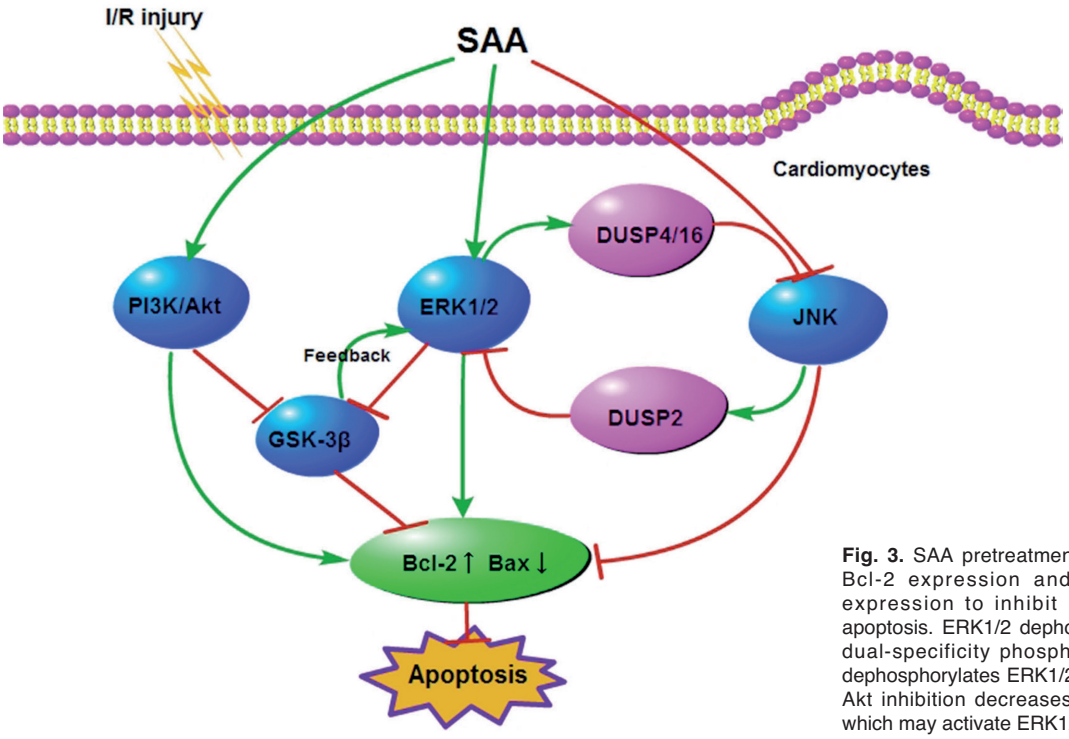


**Fig. 3.** SAA pretreatment increases Akt, ERK1/2, and Bcl-2 expression and decreases JNK and Bax expression to inhibit I/R-induced cardiomyocyte apoptosis. ERK1/2 dephosphorylates JNK by activating dual-specificity phosphatase (DUSP)4/16 and JNK dephosphorylates ERK1/2 by activating DUSP2. PI3K or Akt inhibition decreases phosphorylation of GSK-3β, which may activate ERK1/2 by negative feedback.

cardiomyocytes, suggesting that sustained NF-κB activity contributed to apoptosis (Hamid et al., 2009). SAA has been found to block inflammatory responses by inhibiting the NF-κB pathway (Oh et al., 2011; Chien et al., 2016; Lin et al., 2017). Oh et al. (2011) confirmed that SAA suppressed NF-κB-mediated inflammation in lipopolysaccharide (LPS)-induced RAW264.7 cells by inhibiting the inhibitor of NF-κB kinase subunit beta (IKK-β) . We previously reported that SAA suppressed angiotensin II-induced upregulation of NF-κB expression. A study by Li et al. (2016) found that the protective effects of SAA in murine peritoneal macrophages derived from impairment of NF-κB signaling. SAA pretreatment was also reported to suppress the increase of NF-κB in a skeletal muscle model of I/R injury (Xiang et al., 2018). NF-κB signaling may be active in the mitochondrial apoptosis pathway via the regulation of Bcl-2 expression (Zheng et al., 2016). Few data are available on anti-apoptotic activity of SAA in cardiomyocyte I/R injury that involves inactivation of the NF-κB pathway.

### *SAA, the LOX-1 pathway, and cardiomyocyte apoptosis following I/R*

LOX-1, a lectin-like receptor for oxidized low-density lipoprotein (ox-LDL), was found to be involved in generation of ROS in myocardial I/R injury (Giordano, 2005), and it is upregulated by lipid peroxidation and increased ox-LDL formation, forming a positive feedback loop. LOX-1 is upregulated in I/R cardiomyocyte models, and monoclonal anti-LOX-1 antibodies were found to inhibit apoptosis (Li et al., 2003). Hu et al. (2007, 2008) reported decreased infarct size and less myocardial functional deterioration in LOX-1 knockout mice following I/R injury. The findings provide evidence of LOX-1 involvement in I/R-mediated cardiomyocyte apoptosis. Whether SAA inhibits cardiomyocyte apoptosis by suppressing LOX-1 expression has not been investigated.

## SAA, crosstalk, and cardiomyocyte apoptosis following I/R

The regulation of cardiomyocyte apoptosis involves complex interactions of several signaling pathways. Crosstalk among the pathways forms a signaling network that determines the fate of cardiomyocytes. ERK1/2 and JNK, PI3K/Akt and JNK, and Akt/GSK-3β and ERK1/2 do not act independently of each other. SAA could inhibit myocardial I/R-induced apoptosis via JNK/ERK1/2 crosstalk and JNK/PI3K/Akt crosstalk. Crosstalk between Akt/GSK-3β and ERK1/2 may be also be associated (Fig. 3).

### *SAA, crosstalk between the ERK1/2 and JNK pathways, and cardiomyocyte apoptosis following I/R*

A recent study of the interaction of ERK1/2 and JNK pathways in an isolated rat heart model of I/R-induced cardiomyocyte apoptosis (Fig. 3) found that SAA pretreatment increased p-ERK1/2 and Bcl-2 expression and decreased p-JNK, Bax, and caspase-3 expression. Those effects were blunted by the ERK1/2 inhibitor, PD098059, or the JNK inhibitor, SP600125 (Xu et al., 2014). The interaction of the ERK1/2 and JNK pathways was investigated using DUSP 2/4/16. The results demonstrated that ERK1/2 dephosphorylated JNK in the I/R rat heart model by activating DUSP4/16; furthermore, JNK dephosphorylated ERK1/2 by activating DUSP2. Inhibition of the JNK pathway downregulated DUSP2 activation of ERK1/2 through negative feedback. SAA inhibited myocardial I/R-induced apoptosis by activating ERK1/2 via inhibition of JNK-induced DUSP2 and by inhibiting JNK via activation of ERK1/2 induced-DUSP4/16. The effects were upregulation of ERK1/2 and Bcl-2 expression and downregulation of JNK, Bax, and caspase-3 expression.

### *SAA, crosstalk between the PI3K/Akt and JNK pathways, and cardiomyocyte apoptosis following I/R*

In addition to the studies described above, our group was the first to report on the inhibitory effects of SAA on I/R-induced apoptosis in isolated diabetic rat hearts (Chen et al., 2016). Myocardial I/R increased p-Akt expression in nondiabetic rats, while there was no effect in diabetic rats, which was consistent with the results of previous studies showing that the PI3K/Akt pathway was impaired in diabetic rats. SP600125 or SAA pretreatment increased p-Akt and Bcl-2 expression and decreased p-JNK, Bax, and caspase-3 expression. The results indicate that JNK inhibition restored the PI3K/Akt pathway activity in diabetic rats and that SAA attenuated I/R-induced cardiomyocyte apoptosis by PI3K/Akt pathway activation following the inhibition of JNK.

### *SAA, crosstalk between the Akt/GSK-3β and ERK1/2 pathways, and cardiomyocyte apoptosis following I/R*

Previous studies reported that the activation of ERK1/2 resulted in phosphorylation and inactivation of GSK-3β independent of Akt pathway signaling (Ding et al., 2005; Pal et al., 2017). PI3K or Akt inhibition slightly increased ERK phosphorylation (Pal et al., 2017), this suggested that inhibition of PI3K/Akt decreased phosphorylation and inactivation of GSK-3β, and ERK1/2 may have been activated by negative feedback (Fig. 3). The interaction of the Akt/GSK-3β and ERK1/2 pathways by which SAA mediates its cardioprotective effects against I/R-induced cardiomyocyte apoptosis awaits further investigation.

## Conclusions and perspectives

Advances in cardioprotective and reperfusion therapy have highlighted a need for novel drugs targeting myocardial I/R injury. The available evidence

shows that SAA inhibits I/R-induced cardiomyocyte apoptosis by the PI3K/Akt, GSK-3β, JNK, and ERK1/2 pathways, and by JNK-ERK1/2 crosstalk. The mechanisms of SAA attenuation of cardiomyocyte apoptosis in response to I/R through the P38 MAPK, caspase, JAK/STAT, NF-κB, and LOX-1 signaling pathways needs further clarification. There may be crosstalk between PI3K/Akt and JNK, and Akt/GSK-3β and ERK1/2 during SAA-mediated protection against I/R-induced cardiomyocyte apoptosis. Preclinical data from animal experiments demonstrating the cardioprotective effects of SAA warrant performance of large multicenter clinical trials to determine whether SAA can improve the clinical outcomes of myocardial I/R injury.

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