Myocardial Ischemia-Reperfusion Injury: The Perioperative Application and Cardiac Protective Potential of Ginsenoside Preparations

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ABSTRACT

Aim of review: This review aims to elaborate the effects of ginsenosides in the prevention of myocardial ischemia/reperfusion (MI/R) injury in experimental and clinical studies and further investigate the potential mechanisms contributing to the efficacy of ginsenosides during MI/R procedure.

Method: We searched and reviewed the articles and literatures about the applications of ginsenosides in MI/R processes published in the last two decades.

Recent findings: A large number of pharmacological cardioprotection strategies, such as molecular targeted drugs, were proven efficacy for attenuating MI/R injury in experimental models, however, the efficacy of currently available pharmacological cardioprotection strategies in clinical settings is not convincing. Ginsenoside, the bioactive constituent in ginseng, has been shown to have multiple physiological and pharmacological activities, such as anti-oxidant, anti-inflammation, and anti-apoptosis effects that may serve to combat myocardial injury during MI/R. Different from most currently available drugs that usually target single signaling molecule, ginsenosides possess multiple properties that may prove to be superior in attenuating MI/R injury perioperatively where post-ischemic myocardial injury is severe in elderly patients with pre-existing cardiovascular abnormalities.

Summary: This review discusses current works on the countless pharmacological functions and the potential benefits of ginsenosides in the prevention of MI/R injury. Further large-scale, multi-center clinical researches should be carried out to explore the pharmacological actions of ginsenosides and elucidate whether or not ginsenosides may function better with its multi-target property.

Ischemic heart disease (IHD), one of the major cardiovascular diseases, has been ranked as the leading cause of death globally (1, 2). Reperfusion therapy (e.g., coronary artery bypass graft, percutaneous transluminal coronary angioplasty) is currently the most effective treatment for IHD. However, reperfusion itself may paradoxically cause more serious injury, referred to as "myocardial ischemia-reperfusion (MI/R) injury" (3-5), which is largely a consequence of massive reactive oxygen species (ROS) production. There have been proposed many potentially effective therapies against MI/R injury, such as ischemic or pharmacological pre-conditioning and post-conditioning (6-9). However, taking into account the clinical feasibility, pharmacological intervention has extensive application value compared to the simple ischemic intervention. Over the past three decades, a large number of pharmacological cardio-

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protection strategies, such as molecular targeted drugs (e.g., anti-oxidants, anti-inflammatory agents, magnesium, calcium-channel blockers, glucose-insulin-potassium therapy, adenosine), have been shown to be effective in reducing MI/R injury in experimental animal studies. However, the clinical effects of these pharmacological interventions are neither convincing nor disappointing in the clinical settings of MI/R injury in particular in the cases of cardiac surgeries (10, 11). One major reason is likely that most, if not all, of the molecularly targeted drugs mediate a single signaling regulation pathway as compared with those that may have multiple physiological and pharmacological activities.

Panax ginseng is a well-known traditional herbal medicinal plant for thousands of years in East Asian countries, especially in China. Its root has been taken as a natural invigorant in nourishing and strengthening life, and has been used extensively for treating numerous diseases (i.e. cardiovascular diseases, neurodegenerative disorders, cancer, stroke). Ginsenosides is the bioactive constituents of Panax ginseng that has been shown to have multiple physiological and pharmacological activities, such as anti-oxidant and anti-inflammation properties as well as vasodilator effect. The current general consensus is that oxidative stress and inflammation are the major causes of cardiomyocyte injury induced by ischemia reperfusion. A large number of clinical and epidemiological studies have demonstrated that ginsenosides may confer beneficial effects in attenuating MI/R injury (12-14). This review aims to summarize the available research evidence regarding the therapeutic effects of ginsenosides on MI/R injury and further explore the potential molecular mechanisms.

### Source and Chemistry of Ginsenoside

Ginseng is a perennial plant belonging to the genus Panax of the Araliaceae family (15). A large amount of studies show that the chemical constituents and bioactivities of Panax ginseng mainly include ginsenosides, polysaccharides, peptidoglycans, polyacetylenic alcohols, fatty acids and so on (16-18). It is found that the predominant active constituent of ginseng is ginsenosides. About 150 ginsenosides have been found and isolated from ginseng (19-22). All ginsenosides share a common four-ring hydrophobic structure (23) and are classified into two groups depending on the variability of the sugar number and position: the panaxadiol group (i.e. Rb1 and Rc) and the panaxatriol group (i.e. Rg1 and Re) (24, 25).

### Effects and Mechanisms of Ginsenosides on MI/R Injury

The role of ginsenosides in the prevention and treatment of MI/R has been paid high attention in the field of medicine. Both experimental and clinical evidence have suggested that various ginsenosides can protect cardiomyocytes from I/R injury. This review illustrated the variety of mechanisms contributing to the efficacy of ginsenosides in attenuating MI/R injury as summarized in Figure and described below.

### Anti-Oxidative Effects

ROS are considered molecules that are injurious to biomolecules such as DNA, RNA and proteins as well as membrane lipids, and thus can lead to mitochondria dysfunction and cell death. ROS mediated lipid peroxidation causes disruption of cell membranes and loss of enzyme activity, contributing to cellular damage. It is widely accepted that oxidative stress is the leading cause of MI/R injury (26, 27).

Using a heterotopic heart transplantation model in Wistar rats, Liu K et al. (28) demonstrated that ginsenosides could attenuate global I/R-induced myocardial injury. After administration of ginsenosides at the dose of 80 mg/mL in the St. Thomas II cold cardioplegia, myocardial superoxide dismutase (SOD) activity was significantly higher whereas the malondialdehyde (MDA) content was markedly lower than that in the St. Thomas II cold cardioplegia alone group. In addition, the amounts of oxygen free radicals in the myocardium treated with ginsenosides were also decreased, which suggested that ginsenosides, as a proper additive agent of cardioplegic solution, can protect the myocardium against I/R injury.

Furthermore, another experimental study elu-
elucidate that ginsenosides act as an antioxidant, protecting cardiomyocytes from I/R injury, and this protective effect may be mainly attributable to scavenging oxygen free radical during ischemia and reperfusion (32, 33).

**Anti-Inflammatory Effects**

It is generally known that inflammation response also plays a fundamental role in the progression of MI/R injury (34, 35). Following reperfusion, a large amount of inflammatory cells (including polymorphonuclear neutrophils and monocytes/macrophages) aggregate and cause the release of inflammatory mediators (including cytokines, chemokines, adhesion molecules, and other inflammatory molecules) in the infarcted myocardium (36-38). These inflammatory reactions cause dysfunction of microvascular and cellular metabolism, and finally lead to myocardial damage.

Ginseng total saponin, consisting of various ginsenosides, has been shown to attenuate MI/R injury by increasing the levels of anti-inflammatory molecules such as interleukin-10 (IL-10), and suppressing pro-inflammatory molecules such as IL-1β and IL-6 (31). Furthermore, another study (39) conducted in experimental I/R injury models both in vivo and in vitro evaluated the cardioprotective effects of combinational use of ginsenoside Rg1 and salvianolic acid B (SalB), the main active ingredients of Salviae miltiorrhizae on post-ischemic myocardial injury. Compared with a single dose of SalB or Rg1 alone, SalB-Rg1 combination was more potent to limit myocardial infarct size and down-regulate cytokine secretion including tumor necrosis factor-α (TNF-α) and IL-1β, and suppress the release of soluble vascular cell adhesion molecule-1 (sVCAM-1), a molecule that contributes to the increased vascular permeability induced by I/R injury (33, 40, 41). Moreover, Shenfu injection (SFI, the major components of which are ginsenosides compound) also has been shown to inhibit nuclear factor κB (NF-κB) activity in ischemic and reperfused myocardium and lead to the decrease of pro-inflammatory cytokine expression (42). Ma et al. (43) found that ginsenoside Rb3 decreased the expression of inflammation-related factors, such as monocytic chemotactic protein-1 (MCP-1), MMP-2 and MMP-9 through c-Jun N-terminal kinase (JNK)-mediated NF-κB.
pathway in mouse cardiac myoblast H9c2 cells under oxygen and glucose deprivation. These results suggest that anti-inflammatory property of ginsenosides may be one of the important mechanisms whereby ginsenosides can protect against MI/R injury.

Inhibiting Ca\(^{2+}\) Overload During Ischemia-Reperfusion
Calcium is an important signaling molecule in regulating cell activities and function, such as survival and death. Therefore, modulation of intracellular Ca\(^{2+}\) concentration balance is crucial in maintaining the normal physiological function of cells. Under conditions of cellular injury, such as ischemia and hypoxia, the inhibition of Na\(^{-}\)-K\(^{+}\)-ATPase activity and activation of Ca\(^{2+}\)-ATPase enzyme activity may induce intracellular calcium overload, which leads to mitochondrial damage, cytochrome-c (Cyt-c) release, and activation of caspases pathway, ultimately leading to apoptotic cell death (44-46).

In addition, several studies have showed that the intracellular Na\(^{+}\) overload can induce the reversal of Na\(^{-}\)-Ca\(^{2+}\) exchanger, thus aggravating Ca\(^{2+}\) overload (47). Accordingly, prevention of intracellular Na\(^{+}\) overload is another mechanism to attenuate MI/R injury. Pretreatment with compound K, a metabolite of ginsenosides, has been shown to reduce infarct size in a mouse model of MI/R injury, mainly through inhibition of mitochondrial swelling induced by Ca\(^{2+}\) overload (48). Bai et al. (49) have found that ginsenoside Re can protect the cardiomyocytes against I/R injury by shortening action potential duration and thereby suppressing the L-type Ca\(^{2+}\) current and prohibiting excessive Ca\(^{2+}\) influx in guinea-pig ventricular myocytes. Additionally, Luo et al. (50) have showed that SFI could significantly alleviate post-resuscitation myocardial dysfunction through blocking sodium channels, which can inhibit Na\(^{-}\)-Ca\(^{2+}\) exchanger, and thus, preventing Ca\(^{2+}\) overload.

Anti-Apoptotic Effects
Apoptosis is a physiologic mode of cell death controlled by serial genes, and plays a key role in the process of MI/R injury. It is well known that MI/R-induced cell apoptosis is related to the changes of structure and function of cardiomyocytes induced by overproduction of oxygen free radical and Ca\(^{2+}\) overload. On the other hand, it is associated with the disequilibrium between apoptosis and anti-apoptosis protein expression. Bcl-2/Bax-controlled pathway is the most principal pathway for apoptosis initiation. Down-regulation of Bcl-2/Bax expression leads to the activation of the caspase family of proteases, such as caspase 3, which plays a crucial role in initiating and promoting apoptosis process.

Mechanisms of anti-apoptotic effects of ginsenosides have been evaluated in many studies. Wang et al. (51) employed a rat model of myocardial I/R injury and a neonatal rat cardiomyocyte model of hypoxia-reoxygenation injury, and found that administration of ginsenoside Rg3 significantly reduced myocardial infarct size and decreased the number of early and late phase of apoptotic cell death through inhibiting the activation of the pro-apoptotic genes such as caspase-3 and caspase-9, as well as increasing the ratio of Bcl-2/Bax. Another study reported that pretreatment with ginsenoside Rd also exerted cardioprotective effects both in vivo and in vitro, as evidenced by limited infract size and decreased cellular apoptosis, concomitant with stabilized mitochondrial membrane potential and inhibited cytosolic translocation of mitochondrial Cyt-c via activating protein kinase B (Akt)/glycogen synthase kinase 3\(\beta\) (GSK3\(\beta\)) pathway (52). Other ginsenoside molecules, such as ginsenoside Rg1, Re, Rb1, have also been proved to attenuate MI/R injury by modulating apoptotic-associated pathways (28, 39, 53-55).

Inhibition of Autophagy
Autophagy is a lysosome-dependent degradation pathway which is implicated in various physiological and pathological processes (56). When the body suffers from stress, such as starvation, hypoxia or ischemia, autophagy is activated to maintain intracellular environment hemostasis. However, intense/persistent stress could cause dysregulated or excessive autophagy, which ultimately leads to autophagic cell death (57-59). Therefore, regulation of autophagy plays an important role in protecting myocardium against I/R injury.

A recent study evaluated the effects of ginsenoside Rb1 on autophagy in cardiomyocytes un-
der hypoxia/reoxygenation (H/R) stress (60). The results showed that H/R caused autophagosomes accumulation and decreased cell viability, accompanied with up-regulated expression of pro-autophagic proteins punctate LC3 and Beclin-1 (61). Administration of ginsenoside Rb1 significantly inhibited H/R induced autophagy and apoptosis, which was associated with the increase of cellular ATP content. In addition, ginsenoside Rb1 also modulated autophagy-dependent 5′-adenosine monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway, as well as down-regulated expression of LC3 and Beclin-1. Accordingly, ginsenoside Rb1 is beneficial for the survival of cardiomyocytes under H/R insult via inhibiting autophagy activation.

**Improving Cardiac Energy Metabolism**

Energy metabolism dysfunction is the initial stage of MI/R injury. In view of this, preservation of energy metabolism during I/R insult is regarded as an effective mechanism governing the cardioprotective effects of ginsenosides. The experiment was designed to identify the underlying targets of total ginsenosides of Radix ginseng that are related to the improvement of energy metabolism in an isolated rat heart under I/R insult by using proteomic analysis techniques. The results showed that total ginsenosides of Radix ginseng can markedly increase the protein expressions of LDHB (a subunit of LDH) and ODP-2 (dihydrolipoamide-residue acetyltransferase component of pyruvate dehydrogenase [PDH] complex) which are closely related to the function of tricarboxylic acid cycle (TAC), leading to enhanced cardiac energy metabolism in ischemic rat heart tissue (62). Additionally, pretreatment with notoginsenoside R1 has been confirmed to modulate cardiac energy metabolism in I/R-induced cardiac injury or oxygen and glucose deprivation/reoxygenation (OGD-R) models. Notoginsenoside R1 markedly ameliorated myocardial infarction, improved cardiac function and increased cell viability. More importantly, notoginsenoside R1 prevented energy metabolism abnormality both in vivo and in vitro models, mainly by down-regulating the expression of Rho kinase (ROCK) and up-regulating the expression of the mitochondrial ATP synthase δ-subunit (63). All these results indicate that a variety of ginsenosides can improve energy metabolism and alleviate the degree of reperfusion injury.

**Potential Effects of Ginsenosides on Post-Ishemic Cardiovascular Function**

MI/R injury adversely affects the functioning of heart and vascular mainly due to the impairment of blood supply in the coronary artery. This leads to arrhythmias, cardiac contractile dysfunction and microcirculation obstacle, which eventually leads to the irreversible organ damage in the body (64, 65).

Increasing evidence has proven the ability of ginseng in improving cardiovascular function. Firstly, ginsenosides have been proved valuable for modulating vascular function. Several studies showed that extracts of ginseng could enhance vascular endothelial cell proliferation and migration through the nitric oxide (NO)-, Akt-, extracellular signal-regulated kinases (ERK)- and glucocorticoid receptor (GR)-mediated signaling pathways (66-70). Another study found that potassium channels of vascular smooth muscle cells can be activated by ginsenoside Re through the PI3K/Akt/NO pathways (71). Endothelium regulates blood vessel tone through producing and releasing NO. Secondly, the remodeling of vascular smooth muscle and inhibition of angiotensin II-induced proliferation could be induced by ginsenoside Rg3 (72, 73). Additionally, ginsenoside was reported to effectively suppress platelet aggregation and adherence of leukocytes to the venular wall, and consequently prevent microcirculatory disturbance (74-76). All these biochemical and pharmacological activities of ginsenosides are beneficial for improving cardiac function. Current studies have demonstrated that treatment with ginsenosides facilitated the recovery of hemodynamic parameters and left ventricular function, resulting in increases of coronary flow, cardiac output, left ventricular developed pressure (± dp/dtmax) (31, 52, 77, 78). Moreover, ginsenosides could also ameliorate electrocardiographic abnormality by decreasing I/R-induced increases in QRS interval, QT interval and R-R interval. These results illustrate that ginsenosides can attenuate MI/R injury via improving cardiovascular system function.
So far, a large amount of studies have examined the clinical efficacy of ginsenosides in preventing MI/R injury in patients. Almost all the currently available clinical trials were carried out during cardiopulmonary bypass (CPB). SFI, a well-known traditional Chinese medical formulation containing ginsenoside, has been commonly used in China for the treatment of cardiovascular diseases. Protective effects of ginsenosides extracts in patients of some clinical studies can be seen in table.

Zheng et al. (79) have performed a clinical trial to investigate the effect of SFI on prevention of MI/R injury during CPB. In this study, forty patients scheduled for mitral valve replacement were assigned to the control group and the trial group with different dosages. Compared with the control group, administration of SFI 1.5 ml/kg into CPB priming fluid and pumping 1.5 ml/kg via CPB after aorta declamping significantly decreased the plasma levels of troponin I (cTnI) and, increased SOD activity during CPB process or postoperatively at 24 hours after CPB. Moreover, the rates of atrioventricular block and ventricular arrhythmia were significantly decreased in trial group with SFI dosage of 1.5 ml/kg. Another small sample trial also observed that application of SFI at 1 ml/kg intravenously dripped before induction of anesthesia and 1 ml/kg in priming solution significantly decreased the generation of inflammatory mediator such as IL-6 (80). These results indicate that SFI has a certain protective effect against MI/R injury by decreasing oxidative stress reaction and improving the erythrocyte immune function of patients undergoing CPB.

Another study about one hundred and twenty patients undergoing CPB for congenital heart defects were divided into the placebo control group and SFI group who were treated with 1.35 mg/kg SFI intravenously before and throughout the course of CPB. They found that administration of SFI markedly inhibited inflammatory response and decreased plasma levels of CK-MB, MDA and IL-6, and shortened the time of stay in ICU postoperatively. In addition, well-conducted larger and longer-term studies are carried out to further explore the efficacy of SFI in patients with ischemic cardiomyopathy and acute myocardial infarction with elevated ST segment. Luo et al. (84) carried out a large sample study about one hundred patients to evaluate the efficacy of SFI in treating patients of ischemic cardiomyopathy with heart insufficiency. The patients in the SFI group were given SFI 60 ml once a day and for 10 days each month via intravenous injection. The results showed that treatment with SFI could improve New York Heart Association (NYHA) functional grade and cardiac function by reducing the diameters of left ventricular at the end of diastole and systole, increasing left ventricular ejection fraction, and decreasing plasma N terminal pro-BNP level. Furthermore, patients treated with SFI had lower occurrence of major adverse cardiovascular events (MACE) and mortality. Similarly, in another clinical trial, ninety-seven patients of myocardial infarction were assigned to be treated with conventional therapy and intravenous injection of SFI (0.3 ml/kg or 0.6 ml/kg) once a day additionally. After 14-day therapy, it was observed that the incidence of heart failure was remarkably decreased.

Ginsenosides in the Prevention of MI/R Injury
<table>
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<th>Models</th>
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<th>Reference</th>
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<tbody>
<tr>
<td>VR under CPB</td>
<td>SFI</td>
<td>forty patients</td>
<td>1.0~1.5 ml/kg, IV</td>
<td>before CPB; after initiation of anterior parallel circulation; posterior parallel circulation; at the time of ceasing CPB</td>
<td>the rate of atrioventricular block and ventricular arrhythmia ↓ cTnI and MDA ↓ SOD ↑</td>
<td>Zheng CD et al. (79)</td>
</tr>
<tr>
<td>VR under CPB</td>
<td>SFI</td>
<td>twenty patients</td>
<td>1.0 ml/kg, IV</td>
<td>before induction of anesthesia; 30 minutes CPB; the end of CPB; postoperative 24 hours</td>
<td>MDA ↓ IL-6 ↓ erythrocyte immune function ↑</td>
<td>Tian X et al. (80)</td>
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<tr>
<td>VR under CPB</td>
<td>SFI</td>
<td>one hundred and twenty patients</td>
<td>1.0 ml/kg, IV</td>
<td>during and after operation</td>
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<tr>
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<td>1.35 mg/kg, IV</td>
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<td>SFI</td>
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<tr>
<td>ischemic cardiomyopathy with heart insufficiency</td>
<td>SFI</td>
<td>one hundred patients</td>
<td>60 ml once a day, 10 days each month, IV</td>
<td>before and after treatment</td>
<td>plasma N terminal pro-BNP level ↓ the occurrence of adverse cardiovascular events ↓ the mortality ↓</td>
<td>Luo XY et al. (84)</td>
</tr>
<tr>
<td>acute myocardial infarction</td>
<td>SFI</td>
<td>Ninety-seven patients</td>
<td>0.3 ml/kg, 0.6 ml/kg, IV</td>
<td>14 days after treatment</td>
<td>the incidence of heart failure ↓ Killip grading ↑</td>
<td>Li ZE et al. (85)</td>
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VR, valve replacement; CPB, cardiopulmonary bypass; SOD, superoxide dismutase; MDA, malondialdehyde; IL-6, interleukin-6.
and the Killip grading for the severity of heart failure was significantly reduced in the SFI treated groups (85).

A number of other studies also illustrated that administration of SFI had protective effects not only in cardiovascular system, but also in the brain, gastrointestinal and immune system undergoing CPB (83, 86-88).

Conclusions

A great amount of studies have demonstrated that ginsenosides may confer beneficial effects in preventing MI/R injury. This review summarized mechanistic effects of ginsenosides on oxidative stress, inflammation, apoptosis, energy metabolism and cardiovascular function in MI/R injury using various cellular and animal models. Different from most currently available drugs that usually target single signaling molecule, ginsenosides possess multiple properties that may prove to be superior in attenuating MI/R injury perioperatively where post-ischemic myocardial injury is severe in elderly patients with pre-existing cardiovascular abnormalities. Further large-scale, multi-center clinical researches should be carried out to explore the pharmacological actions of ginsenosides and elucidate whether or not ginsenosides may function better with its multi-target property.

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