Cardioprotection by Ginseng: Experimental and Clinical Evidence and Underlying Mechanisms

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Cardioprotection by Ginseng: Experimental and Clinical Evidence and Underlying Mechanisms

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Abstract

Protection of the ischemic and reperfused myocardium represents a major therapeutic challenge. Translating results from animal studies to the clinical setting has been disappointing, yet the need for effective intervention particularly to limit heart damage following infarction or surgical procedures such as coronary artery bypass grafting is substantial. Among the many compounds touted as cardioprotective agents is ginseng, a medicinal herb belonging to the genus Panax which has been used as a medicinal agent for thousands of years, particularly in Asian societies. The biological actions of ginseng are very complex and reflect composition of many bioactive components although many of the biological and therapeutic effects of ginseng have been attributed to the presence of steroid-like saponins termed ginsenosides. Both ginseng as well as many ginsenosides have been shown to exert cardioprotective properties in experimental models. There is also clinical evidence that traditional Chinese medications containing ginseng exert cardioprotective properties although such clinical evidence is less robust primarily owing to the paucity of large scale clinical trials. Here, we discuss the experimental and clinical evidence for ginseng, ginsenosides and ginseng-containing formulations as cardioprotective agents against ischemic and reperfusion injury. We further discuss potential mechanisms, particularly as these relate to antioxidant properties.
Introduction

Research into cardiac protection has been, and continues to be one of the most, if not the most, active research areas in the cardiovascular field with thousands of papers published. The reason for such high research activity is not difficult to understand since reducing myocardial damage and infarct size following infarction is associated with improved long-term prognosis and reduced risk for the subsequent development of heart failure. Research spanning nearly four decades and encompassing various experimental models (see below) has identified a large number of both pharmacological and non-pharmacological approaches to limit cardiac muscle damage, indeed with a great deal of efficiency at least as seen in experimental studies. Table 1 presents a very limited list of interventions that have shown promise over the years as potentially effective cardioprotective strategies. Unfortunately, robust experimental data have not translated into clinical efficacy, which has recently been referred to as a “remarkable record of failure” (Lefer and Marban 2017). As recently reviewed by Binder et al. (2015) many reasons have been proposed for these disappointing clinical results and the failure to translate animal data to the clinical scenario.

Ginseng has been used as a therapeutic agent for a large number of medical disorders for thousands of years, particularly in Asian societies. Ginseng is a complex natural compound belonging to genus *Panax*, family Araliaceae, represented by various species and containing many bioactive constituents such as the steroid-like saponins named ginsenosides which are thought to be largely but not exclusively responsible for ginseng’s therapeutic properties. Although it is beyond the scope of this review to discuss the general chemical and biochemical properties and characteristics of ginseng species, a number of reviews dealing with these issues can be recommended (Lü et al. 2009; Yamasaki 2000; Yuan et al. 2010; Qi et al. 2011).
Moreover, a recent review focuses on the chemistry, structure, nomenclature and therapeutic properties of individual ginsenosides (Kim et al. 2017). We have recently reviewed in this Journal the potential benefits of ginseng and ginseng-related products to limit myocardial remodelling and heart failure (Karmazyn and Gan 2017). Here, we focus on another potential benefit of ginseng and assess the evidence regarding the direct cardioprotective effects of ginseng, ginsenosides and ginseng-containing products against ischemia and reperfusion induced myocardial injury from both experimental and clinical studies. To fully appreciate the bases for this it is important to understand the experimental models which have been used to identify potentially effective cardioprotective agents as discussed in the following section.

**Experimental models to identify cardioprotective agents**

Both *in vivo* and *in vitro* techniques have been used extensively in the search for identifying potentially effective cardioprotective strategies. The latter are basically the simplest approaches in which isolated cardiomyocytes or cardiac tissues are subjected to ischemic and reperfusion conditions generally by treating these preparations with hypoxic and then oxygenated media with various compositions. As reviewed by Yellon and Hausenloy (2007), the reperfusion component is of paramount importance in view of the well-established concept that reperfusion of the ischemic heart represents an exacerbation and acceleration of already-existing ischemic induced injury. This so-called reperfusion injury has substantial clinical relevance since myocardial reperfusion with various means such as using thrombolytic agents, angioplasty or surgical revascularization represents a critical treatment of patients who have suffered a myocardial infarction (Bassand et al. 2005). Interestingly though, the nature of reperfusion injury appears to be quite distinct from injury observed during the initial ischemic insult and protecting the reperfused heart has also materialized into a major clinical challenge (Yellon and Hausenloy...
Ideally, an effective cardioprotective agent is one which attenuates myocardial injury when administered at the time of reperfusion. Certainly, such treatments would have major clinical usefulness since they can potentially be administered to patients following infarction. Thus, limitation of reperfusion injury represents an important therapeutic target. However, limiting ischemic injury, that is injury occurring prior to reperfusion is also of benefit particularly under circumstances such as coronary artery bypass surgery when effective cardioprotective agents can be administered prior to the surgical procedure therefore protecting the heart during induced ischemia (Mentzer Jr 2011). A summary of the phases of injury occurring during myocardial ischemia and reperfusion is illustrated in Figure 1 in relation to the key role of sodium-hydrogen exchange isoform 1 (NHE1). Activation of NHE1 can lead to intracellular calcium overload through the sodium-calcium exchanger (NCX) resulting in activation of processes that result in apoptotic or necrotic cell death. Thus, timely early reperfusion before the onset of these processes can prevent cell death concomitant with functional recovery (reversible ischemia).

**In vitro models**

The use of isolated cells and tissues *ex vivo* presents several advantages as these models preclude the possible contribution of neuronal and hormonal factors to the injury process thus simplifying the model and providing a better opportunity to study intrinsic mechanisms mediating injury (Maddaford et al. 1999). However, such approaches are also potentially limited in terms of physiological and clinical relevance since they do not represent ischemia and reperfusion *per se* but rather conditions associated with ischemia and reperfusion such as hypoxia and reoxygenation. Nonetheless, Portal et al. (2013) have recently shown that freshly isolated mouse
cardiomyocytes can exhibit a similar profile of injury when subjected to hypoxia and reoxygenation as that seen under in vivo conditions.

More clinically relevant ex vivo models are the isolated freshly perfused heart, the so called Langendorff preparation (Döring 1990) or the Neely working heart (Neely et al. 1967) which are perfused through the coronary arteries, either retrogradely or antegrade respectively, with either simple saline buffers or complex media and which can then be subjected to actual ischemia and reperfusion using various techniques. Even though these experimental models have provided a plethora of valuable information, as with most ex vivo preparations results obtained using these techniques should be interpreted with caution primarily as these preparations are generally devoid of hormonal or neuronal influence or indeed influence by other critical blood-borne factors (Hearse and Sutherland 2000).

In vivo models

As reviewed by Black (2000) cardioprotective strategies can also be studied and assessed using in vivo live animal models. In most cases this involves subjecting animals to coronary artery ligation with reperfusion and determining the modulation of infarct sizes by treatment. Several animal species have been studied using this approach although rats and dogs predominate as animals of choice to determine infarct sizes in vivo. However, with the development of genetic mouse models of heart disease there has been increasing interest in the use of the coronary artery ligation infarction model in this species (Gao et al. 2010). Experimental coronary artery ligation offers an additional advantage as infarct sizes can be readily manipulated simply by altering the duration of the coronary artery ligation period. Moreover, as a general rule the degree of reperfusion injury, that is injury occurring after restoration of coronary flow, is dependent on the duration and therefore the severity of the preceding ischemic period (Figure 1).
As summarized in Table 1, studies using such diverse experimental models have revealed a plethora of experimental approaches including pharmacological agents as well as procedures such as ischemic preconditioning (brief sub-lethal cycles of ischemia prior to prolonged ischemia) and postconditioning (brief cycles of ischemia following prolonged ischemia and before reperfusion) as effective cardioprotective strategies experimentally but these have generally proved disappointing when applied to patients. One of the potential reasons for this is the fact that most experimental animal studies may be poorly applicable to the human population as the former generally employed young animals with no co-existing morbidities, in contrast to what is seen with cardiac patients who are generally in the older age bracket and who often present with other clinical conditions with both factors potentially confounding effective cardioprotection. Another basis for disappointing translational results may lie with the fact that mechanisms of ischemia- and reperfusion-induced cardiac injury are distinct as well as complex. Thus, focussing on the best appropriate target(s) as well as ideal timing of treatment are likely of paramount importance. With respect to the latter this represents a major challenge as administering a cardioprotective strategy after infarction, that is at the time of reperfusion, may have limited value in view of the fact that substantial cell death has already occurred prior to initiation of treatment (Schaper and Schaper 1988). Limiting damage, particularly that produced by reperfusion may therefore represent a challenging goal in the setting of acute myocardial infarction. As alluded to above, a clinical scenario where cardiac protection efficacy could be tested under more controlled environment is in high risk patients undergoing coronary artery bypass grafting (CABG) since in these patients treatment can be initiated during ischemia (i.e. cardiac arrest during surgery) as well as reperfusion following completion of the surgical procedure (Mentzer Jr 2011). Cardioprotection was indeed observed in high risk CABG patients
who were administered the NHE-1 inhibitor cariporide although any potential clinical
development of this agent was negated by the unexpected increased occurrence of
cerebrovascular thromboembolic events (Mentzer et al. 2008).

**Mechanisms of injury**

A complicating factor in designing effective cardioprotective strategies likely also reflects the
complexity of mechanisms underlying injury and cell death. From a general perspective cell
death can occur from both necrosis as well as apoptosis, also referred to as “programmed cell
death” with each mode of death reflecting distinct mechanisms of injury (Fink and Cookson
2005). Although they share some common mechanisms, cardiac injury and cell death occurring
as a consequence of ischemia are quite different and distinct from reperfusion injury occurring
following post-infarction restoration of blood flow. Although it is beyond the scope of this
review to discuss the underlying complex mechanisms of ischemia- and reperfusion-induced
injury in substantial detail various excellent reviews can be recommended (Binder et al. 2015;
Yellon and Hausenloy 2007; Mentzer Jr 2011; Hausenloy and Yellon 2015). Briefly, it is
appropriate to state that defective intracellular ion regulation especially that occurring during
prolonged ischemia is a prerequisite for irreversible ischemic injury, intracellular calcium
dysregulation as evidenced by elevations in intracellular calcium levels represents a major
contributor to this process (Opie 1991). Thus, as illustrated in Figure 1, a major goal of
mitigating ischemic-induced injury involves the inhibition of intracellular calcium overload and
preventing the transition from reversible to irreversible injury (Opie 1991). While reperfusion-
induced injury also reflects complex intracellular events, a major contributing factor to this type
of injury is the rapid reintroduction of oxygen upon early reflow thus leading to the generation of
reactive oxygen species (ROS) resulting in myocyte injury (Maddika et al. 2009;
Braunersreuther and Jaquet 2012). Therefore, inhibitors of ROS generation as well as free radical scavengers have generally proven effective in experimental models of reperfusion injury, both *in vitro* and *in vivo*. The beneficial consequences of inhibiting ROS generation and attenuating intracellular calcium dyshomeostasis are multifold although one of the major consequences which can greatly attenuate the degree of injury is inhibition of the mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane whose activation results in subsequent loss of ionic homeostasis, matrix swelling, outer membrane rupture and the development of apoptosis (Javadov and Karmazyn 2007). Indeed, as will be evident below, inhibition of oxidative stress as well as intracellular calcium abnormalities appear to be some of the primary mechanism underlying the cardioprotective effects of ginseng-related compounds.

Although marked ischemia- and reperfusion-induced injury can be achieved using *in vitro* preparations, it should be noted that mechanisms contributing to such injuries are markedly more complex *in vivo*. This is principally due to the fact that extracardiac factors contribute to myocardial injury as well. A primary example of this, as demonstrated many years ago, would be the accumulation and infiltration of neutrophils in the ischemic area which can then release a myriad of cardiotoxic compounds such as ROS and other pro-inflammatory mediators (Williams 1996; Jordan et al. 1999). Indeed, inhibition of neutrophil accumulation and function represents an effective cardioprotective strategy in the experimental setting.

**Assessment of injury and cardioprotective efficacy**

As will be evident when describing individual examples below, there are a number of determinations one can undertake to assess the degree of injury using the various experimental models that have been referred to. For example, the degree of cardiomyocyte injury can be
determined indirectly by assessing the release of enzymes such as creatine kinase (CK) or lactate dehydrogenase (LDH) as well as Troponin I which can then be measured either in the bloodstream for in vivo studies or in superfusion or perfusion media for ex vivo models of ischemia- and reperfusion-induced injury (Singh et al. 2010). Moreover, injury can be assessed histologically to directly determine the degree of damage or by cell viability assays, the latter of particular usefulness when using myocyte preparations. These approaches do not effectively differentiate between necrotic or apoptotic cell death which have substantially different characteristics and which contribute in their individual ways to mediate myocardial injury (Kajstura et al. 1996). A large number of assays are available which can measure apoptosis by determining the levels of specific enzymes and factors which are involved in the apoptotic process as well as apoptosis-specific injury including DNA fragmentation as determined by TdT-mediated dUTP-biotin nick end-labeling (TUNEL) staining (Darzynkiewicz et al. 2008). Cardiac function is also of substantial benefit as an index of degree of injury or protection by interventions particularly when using experiments involving intact heart preparation either in vivo or in isolated tissues.

**Evidence for ginseng, ginsenosides and ginseng-related products as cardioprotective agents**

**Ginseng species**

The potential cardioprotective properties of ginseng have been known for at least two decades with the finding that Trilinolein, a triacylglycerol purified from *P pseudoginseng*, an atypical member of the *Panax* species, protected the isolated rat heart against ischemia, an effect which was associated with preserved mitochondrial structure, a benefit attributed to antioxidant
properties of this compound as well as maintenance of membrane fluidity and inhibition of calcium influx (Chan et al. 1996, 1997).

Thus, the antioxidant properties of ginseng and its ginsenosides appear to be an important factor in mediating their cardioprotective actions. This has been demonstrated directly in myocytes exposed to a free radical generating system to generate the superoxide anion or by the addition of exogenous ROS and where cell death under these conditions was markedly reduced by berry extracts of *P quinquefolius* or North American ginseng (Shao et al. 2004; Mehendale et al. 2005). Interestingly, in one of these studies (Shao et al. 2004) the efficacy of the *P quinquefolius* berry extract was greater than that seen with extract taken from the ginseng root, suggesting that the former has a greater antioxidant property. It has also been reported (Sui et al. 2001) that *P quinquefolius* leaf extracts, specifically 20s-protopanaxdiol saponins, reduce infarct size through an antioxidant mechanism in dogs subjected to acute myocardial infarction (Sui et al. 2001).

However, numerous other targets may also be involved such as reduction in endoplasmic reticulum stress as shown for *P quinquefolius* which was associated with reduced apoptosis (Xue et al. 2013; Liu et al. 2013, 2015), inhibition of platelet aggregation either when tested alone (Xue et al. 2015) or in combination with established antiplatelet agents (Wang et al. 2015) as well as the regulation of a large number of genes and proteins involved in numerous aspects of myocardial homeostasis as has been proposed for *P notoginseng* as well as ginseng-containing formulations (Yue et al. 2012; Wang et al. 2013; Zhu et al. 2013). Moreover, *P notoginseng* has been shown to enhance mobilization of C-kit+ bone mesenchymal stem cells in rats subjected to myocardial infarction thus potentially enhancing post-infarction myocardial repair (Zhang et al. 2011) as well as to reduce oxidative stress and inflammation (Han et al. 2013). *P ginseng*, also referred to as Asian ginseng, has also been shown to reduced infarct size in a rat infarction model
through a mechanism involving preservation of PI3K and serine/threonine protein kinase Akt (protein kinase B) levels in the myocardium which are important components of the so-called myocardial salvage pathway (PI3K-Akt) resulting in increased NO production via eNOS activation thus representing a potential additional important target site for ginseng-related protection (Pei et al. 2013; Zhou et al. 2011). Interestingly, as discussed below, activation of this pathway has been proposed as a mechanism of action for various individual ginsenosides. Guo et al. (2009, 2010) have proposed an antioxidant effect of P. notoginseng in a rat acute myocardial infarction model which could be due to decreased cytokine production.

Lim et al. (2013, 2014) have reported that Korean red ginseng (P. ginseng subjected to a heating process) administration exerts cardioprotective effects against isoproterenol-induced injury as well as electrophysiological changes in rats in the absence of any changes in heart rate thus implicating a direct cardioprotective effect. In these studies, it was proposed that the salutary effect of Korean red ginseng was due primarily to an antioxidant effect similar to that seen in studies using ischemia models of injury.

The salutary effect of ginseng has also been found to occur in older animals, a potentially important finding as virtually all studies assessing cardioprotection have been carried out using young subjects. For example, Luo et al (2015) have shown that administering a P. ginseng extract for 90 days to aged (18 months old) rats prior to induction of acute coronary artery ligation and reperfusion reduced infarct size, indices of apoptosis, and improved left ventricular function and survival. Several mechanisms were proposed for this protection including the stimulation of the salvage pathway, referred to above as well as two sirtuin isofoms (Sirt1 and Sirt3) which are known to play important roles in cell homeostasis leading overall to reduced apoptosis (Luo et al. 2015).
A summary of the cardioprotective effects of different ginseng species as well as ginsenosides, compound k and ginseng-containing formulations which are discussed in the following sections is presented in Table 2.

**Ginsenosides**

Both total saponin extracts as well as individual ginsenosides have been shown to exert cardioprotective effects. For example, cardioprotection has been documented with individual ginsenosides such as ginsenoside Re which attenuated hydrogen peroxide-induced toxicity in chick cardiomyocytes thus demonstrating an antioxidant property (Xie et al. 2006). However, other mechanisms may also be at play with this particular ginsenoside such as enhancing endothelial nitric production by activating eNOS: it has been shown that this effect is associated with cardiac potassium channel activation thus mitigating ischemia-reperfusion injury (Furukawa et al. 2006). It is interesting that in this study it was shown that ginsenoside Re releases NO via a nongenomic pathway involving sex steroid receptor activation resulting in potassium channel activation in cardiac myocytes (Furukawa et al. 2006). Indeed, ginsenoside Re has been shown to exert several electrophysiological effects on the cardiac cell conducive to cardiac protection in addition to targeting potassium channels including the inhibition of calcium channels, also via increased NO generation (Bai et al. 2003, 2004). In addition, various groups (Wang et al. 2009a; Yin et al. 2005; Xu et al. 2013) have reported that *P quinquefolius* saponin extracts produce a reduction in cardiac injury including an attenuation of apoptosis by directly modifying apoptosis-regulating factors, improving function and decreasing oxidative stress in both myocytes as well as hearts subjected to coronary artery ligation. As noted above, a potential mechanism may also involve the inhibition of mPTP opening. Thus, administering a *P quinquefolius* saponin extract for four weeks to rats prior to ischemia and reperfusion produced a significant reduction in
infarct size associated with mitochondrial protection likely associated with inhibition of mPTP opening (Li et al. 2014). These saponins have also been proposed to exert beneficial effects in infarcted rat hearts by promoting coronary angiogenesis through the upregulation of expression of proangiogenic growth factors (Wang et al. 2007), an effect similarly observed for saponin extracts from the flower buds of *P notoginseng* (Yang et al. 2016).

Several other ginsenosides have also been shown to exert protective effects through a number of proposed mechanisms. For example, Zhu et al. (2009) have shown that ginsenoside Rg1 improved cell viability in cardiomyocytes exposed to hypoxia reoxygenation through an antioxidant effect as well as a reduction in intracellular calcium levels. Rg1 also protected H9c2 cells (a cardiomyoblast cell line) against hypoxia and reoxygenation induced injury through a proposed mechanism involving heme oxygenase-1 (HO-1) upregulation and inhibition of c-Jun N-terminal kinases (JNK) activation (Li et al. 2017a). Deng et al. (2015) showed that Rg1 also improved cardiac function in rats subjected to infarction in which Rg1 was co-administered with salvianolic acid B, extracted from the herb *Salvia miltiorrhiza*, although no benefit was seen in ginsenoside Rb1 treated animals. However, the lack of benefit seen with ginsenoside Rb1 is in contrast with other studies showing robust cardioprotection with Rb1 treatment. Indeed, it is interesting that a single intravenous administration of Rb1 ten minutes before subjecting rats to 45 minutes of coronary artery occlusion and two hours reperfusion reduced cardiac injury suggesting a preconditioning-like effect of this ginsenoside (Wang et al. 2008). The benefit exerted by Rb1 was associated with increased Akt (protein kinase B) phosphorylation, which as already noted is an important component of the so-called myocardial salvage pathway (PI3K-Akt). Moreover, Wang et al. (2008) have reported that Rb1-mediated protection was abolished by wortmannin, a non-specific PI3K inhibitor. Generally similar effects were seen in ischemic
and reperfused hearts from diabetic rats treated with Rb1 (Wu et al. 2011) an effect which may also involve increased production of endothelial-dependent NO via eNOS upregulation (Xia et al. 2011). Improved cardioprotection for both ginsenoside Rb1 and Rg1 was shown to occur when the ginsenosides were administered to rats within core-shell liposomal vehicles in order to improve bioavailability (Zhang et al. 2012). In that study improved protection was seen in both a cerebral model of ischemia and reperfusion as well as myocardial ischemia produced by administering the pituitary gland extract pituitrin, which consists primarily of vasopressin and oxytocin (Zhang et al. 2012). It appears that Rb1 exerts cardioprotective effects through a plethora of mechanisms. For example, in addition to the various mechanisms just noted, ginsenoside Rb1 has recently been proposed to protect the heart by inhibiting succinate accumulation in both ischemic and reperfused working rat hearts as well as hypoxic and reoxygenated cardiomyocytes (Li et al. 2017b). This resulted in improved activity of pyruvate dehydrogenase through inhibition of succinate-associated HIF-1α activation and GPR91 signaling resulting in attenuated cardiac acidification, improved mitochondrial dysfunction and reduced apoptosis (Li et al. 2017b). Cui et al. (2017) also showed that Rb1 exerted several salutary effects in rats subjected to 30 minutes coronary artery ligation followed by 90 minutes of reperfusion including reduced infarct size, apoptosis and improving cardiac function. These effects were attributed to an ability of Rb1 to directly inhibit RhoA thus preventing RhoA-dependent cell signalling and improving ATP preservation. Interestingly, an identical mechanism has been proposed for the cardioprotective effects of ginsenoside Rg1 when administered to rats subjected to 30 minutes of coronary artery occlusion followed by 90 minutes of reperfusion (Li et al. 2018).
Shi and coworkers (2011) demonstrated cardioprotection with the ginsenoside Rb3 which reduced infarct size when administered to rats prior to acute coronary artery occlusion and reperfusion. This salutary effect was associated with reduced indices of oxidative stress in the left ventricle as determined by diminished levels of malondialdehyde and decreased superoxide dismutase activity (Shi et al. 2011).

Ginsenoside Rg5, an important component of heated ginseng has also been shown to increase resistance to hypoxia and reoxygenation induced injury of neonatal rat ventricular myocytes by reducing mitochondrial damage. Yang et al. (2017) have demonstrated that this occurs through the regulation of translocation of two important enzymes, namely hexokinase II, the principal hexokinase isoform in the heart as well as the mitochondrial fission protein Dynamin related protein 1, generally referred to as DRp1.

It is interesting that in a study using a metabonomic approach, a combination of the ginsenosides Rg1 and Rb1 failed to substantially improve myocardial metabolic status when administered to rats for seven days following induction of myocardial infarction (Jiang et al. 2014). However, marked improvement was seen when these ginsenosides were administered in combination with other purported bioactive compounds found in the Chinese Traditional Medicine Sheng-Mai San including schizandrin and ophiopogonin D (Jiang et al. 2014). While the finding of lack of efficacy of the ginsenosides was surprising, the study nonetheless reinforces the concept that ideal cardioprotective benefit of ginseng will likely be particularly evident when used as adjunctive therapy with other medications.

There is good evidence that in vivo treatment with ginseng-related compounds bestows subsequent protection ex vivo. For example, administering either total or purified (panaxadiol or panaxatriol) saponins to rats for seven days resulted in subsequent protection of excised isolated
perfused hearts which were then subjected to ischemia and reperfusion as demonstrated by improved function, attenuation of cell damage concomitant with a reduction in oxidative stress, the latter proposed as the primary basis for the salutary effects of these compounds (Kim and Lee 2010; Aravinthan et al. 2015). Han et al. (2013) showed a similar beneficial effect in rats subjected to coronary artery occlusion and reperfusion that were administered *P. notoginseng*. This study also showed that the benefit could be enhanced with coadministration of extracts of the Safflower plant (*Carthamus tinctorius L*) thus suggesting that combination therapy could effectively enhance the cardioprotective effect of ginseng-related compounds. Furthermore, seven-day pretreatment with a saponin extract from *P. japonicas* exerted substantial protection in rats subjected to 12 hours of coronary artery ligation, without reperfusion, as manifested by improved function, reduced injury including apoptosis as well as reduced markers of oxidative stress and expression levels of cytokines (He et al. 2012; Wei et al. 2014).

It is clear from the evidence that there is substantial variability in terms of efficacy of various ginsenosides as cardioprotective factors. Such variability may reflect the experimental model used or, as recently proposed, the chemical structure of the ginsenoside species. With respect to the latter Feng et al. (2017) recently suggested that compounds lacking the hydroxide group at C6 may be more effective, at least in terms of reducing apoptosis in H9c2 cells (a cardiomyoblast cell line) subjected to hypoxia and reoxygenation although it remains to be determined whether this concept can be applied to other experimental models.

Thus, when taken together it appears that ginsenosides exert salutary effects through multiple mechanisms although many of these ginsenosides share the ability to activate the PI3K-Akt pathway resulting in increased NO production secondary to eNOS activation. How this specifically occurs is not known although as proposed in Figure 2, one possible mechanism may
involve the stimulation of receptor tyrosine kinase (RTK) by individual ginsenosides which results in eNOS activation secondary to stimulation of protein kinase Akt (Fulton et al. 1999; Liu et al. 2014). This hypothesis needs to be assessed with further studies particularly since various ginsenosides such as Rg3 may activate a number of cell signalling pathways which result in eNOS upregulation (Hien et al. 2010).

**Compound K: a bioactive ginsenoside metabolite**

The ginseng metabolite compound K (20-O-(β-D-glucopyranosyl)-20(S)-protopanaxadiol), formed via biotransformation of a number of ginsenosides by intestinal bacteria, also exerts cardioprotective properties. Tsutsumi and coworkers showed that compound K administration to mice reduced infarct size and calcium-induced mitochondrial swelling following ischemia and reperfusion *in vivo* which was associated with enhanced Akt and eNOS activities as discussed above for ginsenoside Rb1 (Tsutsumi et al. 2011). These findings further implicate the involvement of the PI3K-Akt myocardial salvage pathway as a potential key target for the cardioprotective effects of numerous ginseng-related products.

**Ginsenoside-containing preparations**

Complex preparations containing ginsenosides have also been studied as cardioprotective agents, many of which are in common use for the treatment of coronary heart disease in Asian countries (Hung et al. 2015). One of these herbal medications, Shenfu, is currently used in Asian societies, particularly China, as treatment for cardiovascular diseases as well as other conditions. Wang et al. (2009b) have reported that Shenfu reduces apoptosis in cardiomyocytes exposed to hypoxia and reoxygenation, an effect which was associated with increased expression of the antiapoptotic protein B-cell lymphoma 2 (Bcl-2) and a concomitant decrease in pro-apoptotic caspase-3.
activation. Shenfu has also been shown to reduce infarct size when administered to rats prior to induction of myocardial infarction produced by coronary artery ligation followed by reperfusion likely due to antioxidant effects of the formulation (Zheng et al. 2004).

Another similar formulation, Sheng-mei-san, consisting of Radax ginseng plus other constituents including *Fructus schisandrae* has been studied for its cardioprotective properties. Li et al. (1996) have postulated that the presence of lignan-enriched extract of *Fructus schisandrae* accounts for the beneficial effects of Sheng-Mai-San although others have implicated the ginsenoside content of this formulation as a basis for cardioprotection, at least in a mouse model of isoproterenol-induced cardiac injury (Wang et al. 2013). Sheng-Mai-San has also been shown to reduce infarct size in rabbits when administered up to three days prior to infarction, with no benefit observed with acute treatment (Wang et al. 2001). The benefit was proposed to be mediated by activation of protein kinase C and opening of mitochondrial ATP-sensitive potassium channels primarily attributable to the presence of ginsenosides and lignans as the primary active ingredients in this formulation (Wang et al. 2001). Zhao et al. (2016) have reported that a Sheng-mai-san derived product, YiXin-Shu, specifically developed for the treatment of ischemic heart disease, attenuates myocardial ischemic and reperfusion injury in hypercholesterolemic mice through a mechanism involving inhibition of myocardial apoptosis by blunting mitochondrial mediated pro-apoptosis pathways, reduction in oxidative stress as well as by upregulating the nuclear LXRα receptor.

Administering the herbal preparation Danshen, containing notoginseng has also been shown to exert numerous protective effects in rats subjected to acute coronary artery ligation and reperfusion (Ren-an et al. 2014). As for several other ginseng-related products this protection was associated with activation of the Akt myocardial salvage pathway (Ren-an et al. 2014).
Clinical evidence for cardioprotection

As is the case for most areas of ginseng research related to cardiovascular therapeutics, there is a paucity of strong translational evidence demonstrating clinical benefit. In the case of cardioprotection there is some evidence for benefit seen in patients undergoing reperfusion with either thrombolytic therapy (Guo and Zhang 2001) or percutaneous coronary intervention (PCI/angioplasty) (Geng et al. 2004) as demonstrated by reduced injury and improved function in those patients receiving Shenmai. In fact, a meta-analysis of clinical studies in which Shenmai was administered to patients following myocardial infarction showed some benefit in terms of reduced mortality, development of heart failure, circulatory shock and re-infarction (Hu et al. 2012). However, as pointed out by the authors themselves these analyses have serious limitations in view of the small sample size in individual trials and thus should be interpreted cautiously (Hu et al. 2012). In a small study, patients undergoing PCI and receiving Traditional Chinese Medicine consisting of *P. quinquefolius* plus *Salviae miltiorrhizae* in addition to standard medical treatment demonstrated improved left ventricular function compared to standard treatment alone (Qiu et al. 2009). A substantially larger clinical trial has been initiated in December 2014 in China to determine the potential benefit of *P. quinquefolius* in addition to standard medical treatment in patients who have undergone PCI. This multicenter, placebo-controlled, double-blind, randomized controlled clinical trial consisting of 1100 patients administered Xinyue capsules whose major component is *P. quinquefolius* saponins or placebo capsules for twenty-four weeks (Guo et al. 2016). At time of writing, no results from this trial have as yet been reported

Ahn et al. (2011) reported beneficial effects of Korean red ginseng in patients who have suffered an acute myocardial infarction and who were administered the ginseng extract following
coronary stenting. These patients demonstrated improved coronary reserve eight months after
treatment which was associated with increased levels of pro-angiogenic cells as well as reduced
inflammation, thus demonstrating an overall cardioprotective effect (Ahn et al. 2011).

**Conclusion and future directions**

There is a substantial amount of unequivocal experimental data supporting the concept of a
cardioprotective effect of ginseng and ginseng-containing products (summarized in Table 2). As
is the case for many naturally-derived products and ginseng in particular, robust clinical evidence
for the use of ginseng products for protecting the ischemic myocardium is generally lacking in
view of the absence for large scale and well-designed, randomized and placebo-controlled Phase
3 clinical trials. Although challenging to establish for a variety of reasons, such clinical
evaluations are necessary in order to bring ginseng into the widespread clinical arena for cardiac
protection. Unfortunately, the history of translational success of cardioprotective strategies first
shown to be effective in animal studies has been most disappointing (Bolli et al. 2004; Lefer and
Marban 2017). Clearly, substantial work is required in this area in order to fulfil the promise of
ginseng as an effective therapy for the protection of the jeopardized myocardium. In view of the
poor outcomes from cardioprotection clinical studies using single medications it is likely that any
benefit from ginseng will likely be seen when used in combination with other medications and
indeed, various examples of superior efficacy with combination therapy have been cited in this
review. However, this polypharmacy approach does present a challenge particularly in the area
of potential drug interactions which has not been extensively studied *vis-à-vis* ginseng and
commonly used prescription pharmacological agents. Yet interactions between various drugs and
herbal medications including ginseng have been reported, particularly in older individuals
(Agbabiaka et al. 2017). Thus, this represents an important area which needs to be explored in greater detail.
Acknowledgements

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References


Li, J., Yang, Y.L., Li, L.Z., Zhang, L., Liu, Q., Liu, K., et al. 2017b. Succinate accumulation impairs cardiac pyruvate dehydrogenase activity through GRP91-dependent and independent...


Wang, Z., Li, M., Wu, W.K., Tan, H.M., and Geng, D.F. 2008 Ginsenoside Rb1 preconditioning protects against myocardial infarction after regional ischemia and reperfusion by activation of


**Figure 1.** Cascade of events leading to either reversible or irreversible myocardial ischemia after reperfusion. Note that when performed early, reperfusion can lead to myocardial recovery. However, when prolonged further intracellular cellular changes including generation of reactive oxygen species (ROS), elevations in intracellular Ca\(^{2+}\) levels and mitochondrial remodelling can lead to cell death *via* apoptosis or other processes. NHE-1; sodium-hydrogen exchange isoform 1; NCX; sodium-calcium exchange; mPTP; mitochondrial permeability transition pore.

**Figure 2.** Simplified diagram showing how ginsenoside-induced activation of the phosphoinositide 3-kinase (PI3K) Akt/PKB pathway *via* receptor tyrosine kinase (RTK) stimulation can lead to the generation of nitric oxide (NO) resulting in cardiac protection secondary to upregulation of endothelial nitric oxide synthase (eNOS). Please refer to text for details.
Table 1. Examples of some cardioprotective strategies in animal/experimental models

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium channel blockers</td>
<td>Gaviraghi et al. 1995</td>
</tr>
<tr>
<td>Sodium-hydrogen exchange inhibitors</td>
<td>Karmazyn 2013</td>
</tr>
<tr>
<td>Antioxidants/free radical scavengers</td>
<td>Becker 2004</td>
</tr>
<tr>
<td>Eicosanoids (eg prostacyclin)</td>
<td>Szekeres et al. 1991</td>
</tr>
<tr>
<td>Ischemic preconditioning</td>
<td>Stokfisz et al. 2017</td>
</tr>
<tr>
<td>Ischemic postconditioning</td>
<td>Heusch 2015</td>
</tr>
<tr>
<td>Adenosine receptor activation</td>
<td>Donato and Gelpi 2003</td>
</tr>
<tr>
<td>Glucose-insulin-potassium solution</td>
<td>LaDisa et al. 2004</td>
</tr>
<tr>
<td>ATP-sensitive K+ channel activation</td>
<td>Testai et al. 2007</td>
</tr>
<tr>
<td>Nitrates/nitrites</td>
<td>Bryan et al. 2007</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Bice et al. 2016</td>
</tr>
<tr>
<td>Neutrophil inhibitors/depletion</td>
<td>Werns and Lucchesi 1988</td>
</tr>
<tr>
<td>Opioid receptor agonists</td>
<td>Peart et al. 2005</td>
</tr>
<tr>
<td>P2Y receptor activation</td>
<td>Djerada et al. 2017</td>
</tr>
<tr>
<td>RISK* pathway activation</td>
<td>Hausenloy and Yellon 2007</td>
</tr>
<tr>
<td>Stress (heat shock) protein induction</td>
<td>Currie et al. 1988</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>Lavu et al. 2011</td>
</tr>
<tr>
<td>Stem cell therapy</td>
<td>Yu et al. 2017</td>
</tr>
<tr>
<td>Inhibition of microRNAs</td>
<td>Kukreja et al. 2011</td>
</tr>
</tbody>
</table>

*Reperfusion Injury Salvage Kinase
Table 2. Experimental evidence for cardioprotective effects of ginseng, ginsenosides and ginseng-related products in various experimental models

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Treatment</th>
<th>Primary effects</th>
<th>Primary mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated IR rat heart</td>
<td>Trilinolein</td>
<td>Mitochondrial protection</td>
<td>Antioxidant, ↑MF, ↓Ca2+</td>
<td>Chan et al., 1996, 1997</td>
</tr>
<tr>
<td></td>
<td><em>P. ginseng</em></td>
<td>↓injury</td>
<td>Antioxidant</td>
<td>Kim and Lee, 2010</td>
</tr>
<tr>
<td></td>
<td>Ginsenoside Rb1</td>
<td>↓injury</td>
<td>↓succinate accumulation, ↑PDH activity</td>
<td>Li et al., 2017</td>
</tr>
<tr>
<td>ROS treated myocytes</td>
<td><em>P. quinquefolius</em></td>
<td>↓cell death</td>
<td>Antioxidant</td>
<td>Shao et al., 2004; Mehendale et al., 2005</td>
</tr>
<tr>
<td>H2O2 treated myocytes</td>
<td>Ginsenoside Re</td>
<td>↓cell death</td>
<td>Antioxidant</td>
<td>Xie et al., 2006</td>
</tr>
<tr>
<td></td>
<td><em>P. quinquefolius</em></td>
<td>↓cell death</td>
<td>Antioxidant</td>
<td>Xu et al., 2013</td>
</tr>
<tr>
<td>HR myocytes</td>
<td>Ginsenoside Rb1</td>
<td>↓apoptosis</td>
<td>↓succinate accumulation, ↑PDH activity</td>
<td>Li et al., 2017</td>
</tr>
<tr>
<td></td>
<td>Ginsenoside Rg1</td>
<td>↓cell death</td>
<td>Antioxidant, ↓Ca2+</td>
<td>Zhu et al, 2009</td>
</tr>
<tr>
<td>Treatment</td>
<td>Effect on Cell Death</td>
<td>Effect on ER Stress</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td>Ginsenoside Rg5</td>
<td>↓apoptosis</td>
<td>↓mitochondrial damage</td>
<td>Yang et al., 2017</td>
<td></td>
</tr>
<tr>
<td>Shenfu</td>
<td>↓cell death</td>
<td>↓apoptosis</td>
<td>Wang et al., 2009</td>
<td></td>
</tr>
<tr>
<td>TG treated myocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. quinquefolius</em></td>
<td>↓cell death</td>
<td>↓ER stress</td>
<td>Liu et al., 2015</td>
<td></td>
</tr>
<tr>
<td>Canine AMI</td>
<td></td>
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<td></td>
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<tr>
<td><em>P. quinquefolius</em></td>
<td>↓infarct size</td>
<td>Antioxidant</td>
<td>Sui et al. 2001</td>
<td></td>
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<tr>
<td>Rat AMI</td>
<td></td>
<td></td>
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<tr>
<td>EPC</td>
<td>↓oxidative injury</td>
<td>Antioxidant, ↓ER stress</td>
<td>Xue et al., 2013</td>
<td></td>
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<tr>
<td>EPC</td>
<td>↓infarct size</td>
<td>Antiplatelet, anticoagulation</td>
<td>Xue et al., 2015</td>
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<tr>
<td><em>P. quinquefolius</em></td>
<td>↓infarct size, ↑LV function</td>
<td>↓ER stress, ↓apoptosis</td>
<td>Liu et al. 2013</td>
<td></td>
</tr>
<tr>
<td><em>P. quinquefolius</em></td>
<td>↓infarct size</td>
<td>Antioxidant</td>
<td>Wang et al., 2009; Xu et al., 2013</td>
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<tr>
<td><em>P. quinquefolius</em></td>
<td>↓apoptosis</td>
<td>↑Bcl-2, ↓Fas protein</td>
<td>Yin et al., 2005</td>
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<td><em>P. quinquefolius</em></td>
<td>↑micro vessel density</td>
<td>↑angiogenesis</td>
<td>Wang et al., 2007</td>
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<tr>
<td>Xuesaitong</td>
<td>↓infarct size</td>
<td>Numerous protein targets</td>
<td>Wang et al., 2013</td>
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<tr>
<td><em>P. notoginseng</em></td>
<td>↓infarct size</td>
<td>C-kit+ BMSC mobilization</td>
<td>Zhang et al., 2011</td>
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<tr>
<td><em>P. notoginseng</em></td>
<td>↓infarct size</td>
<td>↓oxidative stress, ↓inflammation</td>
<td>Han et al., 2013</td>
<td></td>
</tr>
<tr>
<td>Herb</td>
<td>Effect</td>
<td>Description</td>
<td>Reference</td>
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<tr>
<td>P notoginseng</td>
<td>↓apoptosis</td>
<td>↑angiogenesis</td>
<td>Yang et al., 2016</td>
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<td>P ginseng</td>
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<td>Not determined</td>
<td>Deng et al., 2015</td>
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<td>P japonicas</td>
<td>↓infarct size, ↑LV function</td>
<td>Antioxidant, anti-inflammatory, ↓MAPK</td>
<td>He et al., 2012; Wei et al., 2014</td>
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<td>Rat pituitrin AMI</td>
<td>↓infarct size</td>
<td>Antioxidant</td>
<td>Zhang et al., 2012</td>
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<tr>
<td>Mouse AMI</td>
<td>↓infarct size, ↓arrhythmias</td>
<td>↑PI3K- Akt- eNOS activity (GR/ER-D)</td>
<td>Zhou et al., 2011</td>
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<td>Porcine MI</td>
<td>↓infarct size</td>
<td>↓apoptosis, ↓ER stress</td>
<td>Zhu et al., 2013</td>
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<tr>
<td>Rat IR</td>
<td>↓infarct size</td>
<td>↑cardioprotective proteins</td>
<td>Yue et al., 2012</td>
<td></td>
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<tr>
<td>P quinquefolius</td>
<td>↓apoptosis</td>
<td>↓mPTP opening</td>
<td>Li et al., 2014a</td>
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<tr>
<td>Ginsenoside Rb1</td>
<td>↓infarct size</td>
<td>↑PI3K- Akt- eNOS activity</td>
<td>Wang et al., 2008</td>
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<td></td>
<td>↓infarct size</td>
<td>Cui et al., 2017</td>
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<td>Antioxidant</td>
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<td>↓infarct size</td>
<td>↓RhoA activation</td>
<td>Li et al., 2018</td>
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<td>Treatment</td>
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<td>Outcome</td>
<td>Pathway/Effect</td>
<td>Reference</td>
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<td>Shenfu</td>
<td></td>
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<td>Antioxidant</td>
<td>Zheng et al., 2004</td>
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<td>Danshen</td>
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<td>↓infarct size</td>
<td>↓apoptosis, ↑Akt-eNOS pathway</td>
<td>Ren-an et al., 2014</td>
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<tr>
<td>Diabetic rat IR</td>
<td>Ginsenoside Rb1</td>
<td>↓infarct size</td>
<td>↑PI3K- Akt- eNOS activity</td>
<td>Wu et al., 2011; Xia et al., 2011</td>
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<td>Aged rats IR</td>
<td>P ginseng</td>
<td>↓infarct size, ↑LV function</td>
<td>↑Akt/eNOS, Sirt1 and Sirt3, ↓Erk/caspase 7</td>
<td>Luo et al., 2015</td>
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<tr>
<td>Guinea pig IR</td>
<td>Korean red ginseng</td>
<td>↑function</td>
<td>Antioxidant, anti-inflammatory</td>
<td>Aravinthan et al., 2015</td>
</tr>
<tr>
<td>Rabbit IR</td>
<td>Shreng-mai-san</td>
<td>↓infarct size</td>
<td>↑PKC, ↑mKATP</td>
<td>Wang et al., 2001</td>
</tr>
<tr>
<td>Mouse IR</td>
<td>Compound K</td>
<td>↓infarct size</td>
<td>↑Akt-eNOS pathway</td>
<td>Tsutsumi et al., 2011</td>
</tr>
<tr>
<td>Rat Iso treatment</td>
<td>Korean red ginseng</td>
<td>↓injury, ↑LV function</td>
<td>Antioxidant</td>
<td>Lim et al., 2013</td>
</tr>
<tr>
<td>Porcine Iso treatment</td>
<td>Korean red ginseng</td>
<td>↓injury, ↑LV function</td>
<td>Antioxidant</td>
<td>Lim et al., 2014</td>
</tr>
<tr>
<td>Mouse Iso treatment</td>
<td>Sheng-mai-san</td>
<td>↓injury</td>
<td>Antioxidant</td>
<td>Wang et al., 2013</td>
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</table>

AMI, acute myocardial infarction; ROS, reactive oxygen species; MF, membrane fluidity; TG, thapsigargin; ER, endoplasmic reticulum; IR, ischemia + reperfusion; EPC, *Panax quinquefolius* + Corydalis tuber (also known as Shenyuan); LV, left ventricle; BMSC, bone mesenchymal stem cells; ARD, androgen receptor dependent; GR/ER-D, glucocorticoid and/or estrogenreceptor-dependent; Iso, isoproterenol; mPTP, mitochondrial permeability transition pore; PDH, pyruvate dehydrogenase; PNS-HLV, *Panax notoginsenoside*-loaded core-shell hybrid liposomal vesicles; PKC, protein kinase C; mKATP, mitochondrial ATP-sensitive potassium channels
Intracellular acidosis

NHE-1 activation

Reverse-mode NCX activation

Increased intracellular Ca\(^{2+}\)

mPTP opening

Reperfusion (ROS, Ca\(^{2+}\))

Release of pro-apoptotic and pro-necrotic factors from mitochondria

CELL DEATH

RECOVERY

REPERFUSION INJURY