Themed Section: Redox Biology and Oxidative Stress in Health and Disease

REVIEW ARTICLE

Effect of hypercholesterolaemia on myocardial function, ischaemia–reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning

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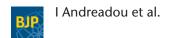
Hypercholesterolaemia is considered to be a principle risk factor for cardiovascular disease, having direct negative effects on the myocardium itself, in addition to the development of atherosclerosis. Since hypercholesterolaemia affects the global cardiac gene expression profile, among many other factors, it results in increased myocardial oxidative stress, mitochondrial dysfunction and inflammation triggered apoptosis, all of which may account for myocardial dysfunction and increased susceptibility of the myocardium to infarction. In addition, numerous experimental and clinical studies have revealed that hyperlcholesterolaemia may interfere with the cardioprotective potential of conditioning mechanisms. Although not fully elucidated, the underlying mechanisms for the lost cardioprotection in hypercholesterolaemic animals have been reported to involve dysregulation of the endothelial NOS-cGMP, reperfusion injury salvage kinase, peroxynitrite-MMP2 signalling pathways, modulation of ATP-sensitive potassium channels and apoptotic pathways. In this review article, we summarize the current knowledge on the effect of hypercholesterolaemia on the non-ischaemic and ischaemic heart as well as on the cardioprotection induced by drugs or ischaemic preconditioning, postconditioning and remote conditioning. Future perspectives concerning the mechanisms and the design of preclinical and clinical trials are highlighted.

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Abbreviations

AMI, acute myocardial infarction; BH4, tetrahydrobiopterin; CAD, coronary artery disease; eNOS, endothelial NOS; iNOS, inducible NOS; LV, left ventricular; MPT, mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; PC, ischaemic preconditioning; PostC, postconditioning; RAS, renin-angiotensin system; RIPC, remote preconditioning; RISK, reperfusion injury salvage kinase

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TARGETS	
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GPCRs[b]	ERK2
AT$_1$ receptor	GSK3β
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Cyclosporine A (CsA)	VCAM-1
Fasudil	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e,f]Alexander *et al.*, 2015a,b,c,d,e,f).

Introduction

Although cholesterol is one of the main constituents of cellular membranes and plays an important role in hormone and bile acid synthesis, increased circulating levels, especially if oxidized, are detrimental resulting in atherosclerosis and thus in the development and progression of coronary, carotid and peripheral vessels' disease (Félix-Redondo *et al.,* 2013). Several years ago, clinical studies clearly showed a linear relation between the regression of cholesterol levels and the consequent improvement of clinical outcome in patients suffering from hypercholesterolaemia (Castelli *et al.,* 1989; Rubin *et al.,* 1990). Hypercholesterolaemia is widely accepted as a principal risk factor for coronary artery disease (CAD) (Tiwari and Khokhar 2014), and patients with extremely increased levels of cholesterol have an elevated risk of ischaemic events regardless of their genotype (Sniderman, *et al.,* 2014). Moreover, analysis of the Kaiser Permanente Heart Study and Framingham Heart Study cohorts showed significant associations between cholesterol levels and the risk for cardiovascular mortality in individuals with and without a history of CAD (Castelli *et al.,* 1989; Rubin *et al.,* 1990; Wong *et al.,* 1991).

Although, the observations from clinical trials and experimental studies suggest an effect of cholesterol on myocardial function (Huang *et al.,* 2004), little is known about its effects on cardiac function apart from CAD. Hypercholesterolaemia has been proposed to have direct negative effects on the myocardium itself, in addition to the development of atherosclerosis, with several studies demonstrating increased myocardial injury in hypercholesterolaemic animals (Hoshida *et al.,* 1996a; Ferdinandy *et al.,* 1998a; Scalia *et al.,* 2001; see for review by Ferdinandy, 2003; Osipov *et al.,* 2009). Hypercholesterolaemia alone increased myocardial necrosis by 45% over what was observed in normal fed animals (Osipov *et al.,* 2009), and impaired diastolic function *in vitro* as well as *in vivo* (Onody *et al.,* 2003; Huang *et al.,* 2004; Varga *et al.,*

2013). Intracellular lipid accumulation in cardiomyocytes and several alterations in the structural and functional properties of the myocardium have been observed in response to a high cholesterol diet in rodents (Onody *et al.,* 2003; Puskas *et al.,* 2004). Thus, it seems that hypercholesterolaemia is not only detrimental for the vasculature but is also a risk factor for increased cariomyocyte death and poor left ventricular (LV) systolic function in patients following acute myocardial infarction (AMI) (Corti *et al.,* 2001). However, the molecular mechanisms by which chronically elevated cholesterol can detrimentally affect the cardiomyocyte are poorly understood.

In this review article, we summarize the current knowledge on the effect of hypercholesterolaemia on the non-ischaemic and ischaemic heart, as well as on cardioprotection induced by ischaemic preconditioning (PC), postconditioning (PostC) and remote conditioning. Additionally, we summarize the effects of hypercholesterolaemia on drug-induced cardioprotection as well as the effect of antihyperlipidaemic drugs on cardioprotection. Future perspectives concerning the mechanisms and the design of preclinical and clinical trials are highlighted.

Effect of hypercholesterolaemia on the myocardium

Hypercholesterolaemia causes endothelial dysfunction

Numerous animal and clinical models have reported impaired endothelium-dependent and independent relaxation in the presence of hypercholesterolaemia (Kawashima and Yokoyama, 2004). Both acute and chronic elevations in blood cholesterol induce nitro-oxidative stress in microvascular endothelium that results from an increased generation of ROS and a decreased bioavailability of NO (Davidson, 2010), up-

regulated inflammation (Liu *et al.*, 2009), inhibition of NOS (Prasan *et al.*, 2007), and increased cardiomyocyte apoptosis (Wang *et al.,* 2002).

ROS can be produced by a variety of cells that have been implicated in the inflammatory responses to hypercholesterolaemia, such as neutrophils, monocytes, B-lymphocytes, platelets, mast cells, endothelial cells and vascular smooth muscle cells (Stokes *et al.*, 2002). Potential sources of ROS in endothelial cells that have been identified so far, include NADPH oxidase (Konior *et al.*, 2014), xanthine oxidase, enzymes involved in the metabolism of arachidonic acid (lipoxygenase and cyclooxygenase) and NO synthases (NOS) (reviewed in Stokes *et al.*, 2002). One of the most important oxygen free radicals that is produced during hypercholesterolaemia is superoxide anion (Landmesser *et al.*, 2000; Napoli and Lerman, 2001). The premise that this enhanced superoxide anion production is due to the elevated blood cholesterol level is further supported by the observation that dietary correction of the hypercholesterolaemia restores superoxide production to normal levels in isolated arterial vessels (Ohara *et al.*, 1995).

Both the expression and activity of NADPH oxidase is responsible, at least in part, for the increased superoxide anion production in cholesterol-fed apolipoprotein B100 transgenic mice (Csont *et al.*, 2007), and in postcapillary venules in skeletal muscle of hypercholesterolaemic wildtype or p47phox$^{-/-}$ mice (Stokes *et al.*, 2001). The enhanced superoxide anion production in arterial vessels from hypercholesterolaemic rabbits was blunted by treatment with either allopurinol (a xanthine oxidase inhibitor) or heparin, which competes with xanthine oxidase for binding to sulfated glycosaminoglycans on endothelial cells (Landmesser *et al.*, 2000; Napoli and Lerman, 2001). Supporting the role of xanthine oxidase in ROS formation in hypercholesterolaemia, superoxide generation by endothelial NOS (eNOS) occurs as a result of uncoupling of L-arginine metabolism from NO production and a reduction in the eNOS cofactor tetrahydrobiopterin (BH4) (Cosentino and Katusic, 1995; Vergnani *et al.*, 2000). Furthermore, an increased formation of peroxynitrite, a toxic reaction product of superoxide and NO, has been observed in hyperlcholesterolaemic rat myocardium, and it is accompanied by a decrease in the bioavailability of NO (Onody *et al.*, 2003). Peroxynitrite induces DNA damage, increases lipid peroxidation, and causes post-translational modification of proteins (e.g. nitration and oxidation of thiol groups) (Pacher *et al.*, 2005), contributing to the development of cardiac dysfunction observed in hyperlcholesterolaemic rats.

The above observations in experimental studies have been confirmed in humans; an increased NADPH oxidase-mediated superoxide anion generation was observed in vessels of hypercholesterolaemic patients (Assmann *et al.*, 1996; Guzik *et al.*, 2000; Stokes *et al.*, 2001; Itoh *et al.*, 2002).

One additional potential mechanism by which hypercholesterolaemia-derived oxidative stress could induce cardiac myocyte dysfunction and death is through disruption of mitochondrial function. Increased oxidative stress during hypercholesterolaemia enhances the mitochondrial permeability transition (MPT) response. MPT dissipates the proton electrochemical gradient ($\Delta\Psi_m$), leading to ATP depletion, further ROS production, swelling and rupture of the mitochondria, thereby releasing pro-apoptotic and pro-death proteins into the cytosol (Halestrap, 2009). Hypercholesterolaemia increases mitochondrial oxidative stress and enhances the MPT response in the porcine myocardium (McCommis *et al.*, 2011).

In conclusion, when blood cholesterol concentrations are elevated, a low-grade systemic inflammatory response is elicited in multiple vascular beds and may create an environment in the extracellular compartment, possibly through the generation of cytokines, oxidized molecules, for example, oxidized LDL, and other inflammatory mediators, that predisposes the endothelial cells of large arteries to an inflammatory phenotype. Inflammation is associated with increased ROS production that may overcome cellular defence mechanisms leading to atherogenesis, protein damage and enzyme inactivation, and eventually to loss of contractile function and vascular dysfunction (Misra *et al.*, 2009), which may also account for the increased susceptibility of the myocardium to ischaemia–reperfusion injury and infarction.

Hypercholesterolaemia affects cardiac gene expression profile

Hypercholesterolaemia has been shown to affect the global cardiac gene expression profile at the mRNA level in several studies. In an early study, Puskas *et al.* reported that in the hearts of rats on a cholesterol-enriched diet the expression of numerous genes were modulated, including those involved in energy metabolism, heat shock proteins, ion channels and structural proteins (Puskas *et al.*, 2004). Similar results were found in Zucker Diabetic Fatty (ZDF) animals that also show a hypercholesterolaemic profile (Sárközy *et al.*, 2013). Hypercholesterolaemia has also been shown to affect cardiac microRNA profile leading to increased NOX-4 expression and nitro-oxidative stress (Varga *et al.*, 2013). These results indicate that hypercholesterolaemia dramatically changes cardiac transcriptomics affecting several known and yet unknown cellular signalling pathways that may impact cardiac function *per se* and the susceptibility of the heart to ischaemia/reperfusion injury (see for a recent review: Varga *et al.* 2015). Systematic analysis of the above mentioned large scale transcriptomic data and more 'omics' data generated in future studies will be necessary to explore alterations in the cellular signalling network of the hypercholesterolaemic heart in comparison with those of the normal heart (Varga *et al.* 2015; Perrino *et al.*, 2017).

Effect of hypercholesterolaemia on myocardial ischaemia/reperfusion injury and cardioportection

Effect of hypercholesterolaemia on myocardial ischaemia/reperfusion injury

Larger myocardial infarct sizes have an ominous long-term prognosis compared to the smaller ones with increased morbidity and mortality. Restriction of the final infarct size is therefore mandatory for the health status and the future of the patients suffering from AMI. How the presence of hypercholesterolaemia affects infarct size is not clear, as the

results that exist in the literature in different species and models of hypercholesterolaemia are contradictory. In this regard, larger infarctions have been described within the first hours after acute ischaemia and reperfusion in cholesterol-fed pigs (Osipov *et al.*, 2009) and rabbits (Golino *et al.*, 1987). In rat isolated hearts exposed to hypercholesterolaemia, induced by a cholesterol enriched diet, the final infarct size and the release of myocardial biomarkers of necrosis and apoptosis were significantly increased, while the recovery of LV function was not affected (Wu *et al.*, 2015b). In contrast, the infarct size was similar in hypercholesterolaemic and normocholesterolaemic rats, but the LV remodelling and risk of developing heart failure were worse in the animals fed the hypercholesterolaemic diet (Maczewski and Maczewska, 2006). New Zealand White rabbits, fed for 4-weeks with a hypercholesterolaemic diet and subjected to 30 min myocardial ischaemia and 2 h reperfusion, exhibited a significantly increased infarct size compared with animals fed a normal diet (Hoshida *et al.*, 1996b; Jung *et al.,* 2004). Additionally, greater cardiac damage, as compared with normal-fed rabbits, was also observed in cholesterol-fed rabbits that were subjected to 60 min of myocardial ischaemia followed by 60 min of reperfusion (Ma *et al.*, 1996).

In contrast, using a model of 30 min ischaemia and 3 h reperfusion a similar degree of myocardial infarction was observed in cholesterol-fed and normal fed rabbits (Andreadou *et al.*, 2006; 2012; 2016; Iliodromitis *et al.*, 2006; 2010). Many other studies have also shown similar infarct sizes in normal and cholesterol-fed rats with no presence of significant atherosclerosis (Giricz *et al.*, 2009; Görbe *et al.*, 2011; Csont *et al.,* 2013; Csonka *et al.*, 2014). These divergent results are possibly related to different species, diet and experimental protocols. However, it can be concluded that the majority of the studies show that hypercholesterolaemia increases infarct size to some extent in animal models.

The mechanism of the effect of hypercholesterolaemia on myocardial ischaemia–reperfusion injury is still not well understood. It has been demonstrated that decreased cardiac NO content, increased oxidative/nitrosative stress, enhanced apoptotic cell death and dramatic changes in the cardiac gene expression profile, as a consequence of hypercholesterolaemia (see for earlier review: Ferdinandy, 2003; Ferdinandy *et al.*, 2007), may play an essential role in the manipulation of myocardial ischaemia/reperfusion injury in the presence of hypercholesterolaemia. Hypercholesterolaemia decreases the bioavailability of NO with a down-regulation of eNOS, in association with increased production of oxygen-derived free radicals that may inactivate NO. More specifically, increased plasma LDL inhibits the active transport of L-arginine by endothelial cells, uncoupling the eNOS pathway, hence limiting NO synthesis and leading to superoxide anion production (Pritchard *et al.*, 1995; Wilson *et al.,* 2001). The contribution of increased nitrotyrosine formation to the development of atherosclerosis and thus to CAD has been demonstrated in patients with hypercholesterolaemia in combination with CAD (Shishehbor *et al.*, 2003). Experimental hypercholesterolaemia is associated with increased myocardial oxidative stress and inflammation, attenuation of cell survival pathways and the induction of apoptosis (Wang *et al.*, 2002; Osipov *et al.*, 2009).

Additionally, it should be noted that the extent of myocardial injury after ischaemia/reperfusion is determined not only by the tolerance of cardiomyocytes to ischaemia/reperfusion injury but also by the coronary collateral blood flow and rate-pressure product at the time of myocardial ischaemia (Reimer *et al.*, 1985). LDL up-regulates the renin-angiotensin system (RAS), leading to increased generation of angiotensin II, which in turn, binds to the type 1 angiotensin II receptor (AT_1 receptor) and activates a signalling cascade that results in the enhanced accumulation of the cholesteryl ester (Rafatian *et al.*, 2013). In particular, hypercholesterolaemia seems to promote the up-regulation of AT_1 receptor genes followed by the structural overexpression of vascular AT_1 receptors for angiotensin II (Borghi *et al.*, 2016). Studies in rabbits showed that hypercholesterolaemia increased cardiac diastolic pressure after 1 month of cholesterol treatment, and this was correlated with increased levels of superoxide in the aortas, and to a higher expression of NADPH subunits, associated with altered vasorelaxation (Collin *et al.*, 2007). Clinical studies have also suggested a role of some lipoprotein subfractions as risk factors for the development of hypertension (see for a recent review: Borghi *et al.*, 2016). The development of vascular damage in patients with hypercholesterolaemia could also involve the activation of the RAS, and although the mechanisms of interaction between hypercholesterolaemia and hypertension have not been completely elucidated, there is growing evidence that the involvement of RAS can be considered as a common link between hypertension and hypercholesterolaemia.

Effect of hypercholesterolaemia on cardioprotection induced by ischaemic preconditioning

Initially, hypercholesterolaemia was shown to alter responses to ischaemic preconditioning (PC); pacing-induced PC was blocked in hypercholesterolaemic rabbits and rats (Szilvassy *et al.*, 1995). This loss of pacing-induced PC was further noted in another experimental model, in rats administered a cholesterol-enriched diet for 24 weeks (Ferdinandy *et al.*, 1997). Furthermore, isolated papillary muscle from rats fed a high-fat diet was more susceptible to the effects of ischaemia and less protected by the effects of PC compared with controls (Kocić *et al.,* 1999). Although studies performed in mice and rats showed consistent results, divergent results have been observed in anaesthetised rabbits. The infarct size-limiting effect of one cycle PC (5 min occlusion and 10 min reperfusion) was shown to be blunted in the hypercholesterolaemic (16 weeks) rabbit heart subjected to ischaemia/reperfusion (Ueda *et al.*, 1999), whereas hypercholesterolaemia (8 weeks) did not attenuate the reduction in myocardial infarction in rabbits subjected to one cycle of PC, comprising 5 min of regional ischaemia plus 10 min reperfusion (Kremastinos *et al.*, 2000). The difference between the above protocols was the duration of cholesterol feeding, that is, 16 versus 8 weeks. Similar results were obtained in a later study, using two cycles of 5 min ischaemia followed by 10 min reperfusion before sustained ischaemia as a PC stimulus, corroborating the finding that PC limits the infarct size in hypercholesterolaemic animals (Iliodromitis *et al.*, 2006). Consistent with the

latter results, Jung *et al.* showed that hypercholesterolaemia did not affect the beneficial influence of PC on infarct mass (Jung *et al.*, 2000).

In patients with hypercholesterolaemia, an early cardiomyopathy characterized by systolic and diastolic dysfunction has been observed, producing a substratum for an 'impaired preconditioning' (Talini *et al.*, 2008). In this respect, two clinical studies investigated the effect of conditioning interventions in the context of hypercholesterolaemia. Both studies examined the effects of repeated balloon inflations at the time of angioplasty in patients with CAD and demonstrated that hypercholesterolaemia attenuated the anti-ischaemic effect of preconditioning during coronary angioplasty (Kyriakides *et al.*, 2002; Ungi *et al.*, 2005). Moreover, hypercholesterolaemia accelerated the development of intracoronary ST-segment elevation in humans (Ungi *et al.*, 2005).

In summary, divergent results exist in the literature concerning cardioprotection by PC in the presence of hypercholesterolaemia. While shorter durations of hypercholesterolaemia may not affect the cardioprotective signalling of PC, longer durations may disrupt this cardioprotective effect. This was observed in animal studies and has been confirmed in clinical trials.

Mechanisms of the interaction between ischaemic preconditioning and hypercholesterolaemia

Several potential mechanisms have been proposed to explain the lack of a PC effect in hypercholesterolaemia. Since hypercholesterolaemia is linked to oxidative/nitrosative stress in the vasculature and in the myocardium (Szilvassy *et al.*, 2001; Giricz *et al.,* 2006) and to a decreased NO bioavailability, many studies have focused on the role of NO as a potential mechanism of PC's lost effect. A low concentration of NO is associated with a high concentration of asymmetric dimethylarginine (ADMA), and it has been shown that during hypercholesterolaemia the level of ADMA is elevated and the cardioprotective effects of PC are eliminated in rats (Landim *et al.*, 2013). The beneficial effects of late PC were shown to be abolished in an *in vivo* rabbit model of hypercholesterolaemia, due to an impaired up-regulation of BH4, which is essential for inducible NOS (iNOS) (Tang *et al.*, 2005).

With regard to the involvement of apoptosis signalling in the effects of PC, it has been shown that hypercholesterolaemia prevents the effects of sevoflurane-induced PC by altering the upstream signalling of glycogen synthase kinase 3 β (GSK3β) indicating that acute GSK inhibition may provide a novel therapeutic strategy to protect hypercholesterolaemic hearts against ischaemia/reperfusion injury (Ma *et al.*, 2013). In isolated hearts from cholesterol-fed rats, the protective effect of PC mediated by moderate inhibition of MMP2 was blocked, whereas a reduction in infarct size could be produced using an MMP inhibitor in non-preconditioned hearts (Giricz *et al.*, 2006; Bencsik *et al.*, 2014). Hypercholesterolaemia also causes an alteration in one of the main signal transduction elements of the conditioning mechanism, connexin, producing a redistribution of the intracellular localization of connexin 43 in the cardiomyocytes, and this might be a potential explanation for the loss of the myocardial

infarction-limiting effect of PC in the presence of hypercholesterolaemia (Görbe *et al.*, 2011).

The effects of PC in ischaemia/reperfusion injury during hypercholesterolaemia and the proposed mechanisms are summarized in Table 1.

Effect of hypercholesterolaemia on cardioprotection induced by ischaemic postconditioning

PostC triggered by two different algorithms (six cycles of 10 s ischaemia separated by 10 s reperfusion, and four cycles of 30 s ischaemia separated by 30 s reperfusion immediately after the end of the index ischaemia) has been found to be ineffective at limiting the infarct size in anaesthetised rabbits with hypercholesterolaemia and atherosclerosis (Iliodromitis *et al.*, 2006). This initial observation was further confirmed in mini swines (Zhao *et al.*, 2007) and in hypercholesterolaemic rats (Kupai *et al.*, 2009). In contrast to the above findings, in rabbit isolated crystalloid-perfused hearts, PostC reduced the infarct size in hypercholesterolaemic animals (Donato *et al.*, 2007) and the same result was observed in hypercholesterolaemic rats (Zhao *et al.*, 2009). These results may show that some components of the hypercholesterolaemic blood contributes to the attenuation of the effectiveness of PostC in hypercholesterolaemia.

In summary, although studies are not consistent, most of the robust studies showed that cardioprotection by PostC is abolished in the presence of hypercholesterolaemia, indicating that hypercholesterolaemia interferes with the molecular signalling of PostC.

Mechanisms of the interaction between ischaemic postconditioning and hypercholesterolaemia

NO pathway and nitro-oxidative stress. The myocardial NO-cGMP pathway seems to be impaired in hypercholesterolaemia. In hypercholesterolaemic rats, phosphorylation of eNOS and Akt was decreased compared with controls, which may result in a decrease in NO production, and the loss of effect of cardioprotective interventions (Penumathsa *et al.,* 2007). Similar results and a decreased phosphorylation of eNOS were found in hypercholesterolaemic rabbits (Andreadou *et al.*, 2012). Similar to the findings mentioned above for PC, a low concentration of NO is associated with a high concentration of ADMA, hypercholesterolaemia elevated ADMA and eliminated the cardioprotective effects of PostC in rats. It has also been shown that although hypercholesterolaemia did not modulate the basal expression of PKG, its oxidized dimeric form was more abundant in hearts of hypercholesterolaemic animals possibly due to increased oxidative stress (Giricz *et al.*, 2009). It is interesting to note that PostC increased cardiac 3-nitrotyrosine concentrations in the normally fed rats but not in the cholesterol-fed group, when measured at the fifth minute of reperfusion, indicating that an early increase in peroxynitrite after PostC plays a role in cardioprotection (Kupai *et al.*, 2009). In contrast, in rabbit myocardium, PostC reduced nitrotyrosine concentrations in the normal fed group but not in the cholesterol-fed group at the 10th minute of reperfusion,

Table 1

Effect of hypercholesterolaemia (HC) on cardioprotection induced by ischaemic and pharmacological PC

Experimental model	Effect on PC	Proposed mechanism(s)	Reference
Isolated rat hearts subjected to PC	Elimination of infarct size reduction by PC	HC abolished PC-induced inhibition of myocardial MMP2 activation and release	Giricz et al., 2009
Isolated rat hearts subjected to PC	Elimination of infarct size reduction by PC	Loss of cardioprotection by PC in HC is associated with a redistribution of both sarcolemmal and mitochondrial connexin 43	Görbe et al., 2011
Rats exposed to pacing-induced PC	Elimination of pacing-induced cardioprotection by PC	HC induced deterioration of cardiac NO metabolism	Ferdinandy et al., 1997
Rats subjected to three cycles of PC	Elimination of infarct size reduction by PC	HC elevated ADMA and eliminated the cardioprotective effects of PC	Landim et al., 2013
Rabbits exposed to pacing-induced PC	Elimination of pacing-induced cardioprotection by PC	HC impaired cardiac NO synthesis	Szilvassy et al., 1995; Ferdinandy et al., 1998a,b
Rabbits subjected to one cycle PC	Elimination of the infarct size-limiting effect of PC	HC prevented ecto-5′-nucleotidase activation by PC	Ueda et al., 1999
Rabbits subjected to one cycle of PC	Myocardial infarction reduction by PC was not attenuated by HC	Observational study	Kremastinos et al., 2000
Rabbits subjected to two cycles of PC	Myocardial infarction reduction by PC was not attenuated by HC	Observational study	Iliodromitis et al., 2006
Rabbits subjected to one cycle of PC	Myocardial infarction reduction by PC was not attenuated by HC	Reduced calcium-ionophore stimulated endothelial NO-release were found in isolated aortic rings of hypercholesterolemic animals suggesting that NO produced by the endothelium is not a prime factor in the cardioprotective mechanism of PC	Jung et al., 2000
Rabbits subjected to late PC	Elimination of infarct size reduction by late PC	Impaired up-regulation of BH4, which is essential for inducible nitric oxide (NO) synthase	Tang et al., 2005
Sevoflurane-induced PC in rats	Elimination of sevoflurane-induced cardioprotection	HC altered the upstream signalling of GSK3β	Ma et al., 2013
Sevoflurane-induced PC in rats	Elimination of sevoflurane-induced cardioprotection	Interference with the iNOS/mitochondrial ATP-dependent K$^+$ channel pathway	Zhang et al., 2012
Fasudil induced pharmacological PC in rats	Low-dose fasudil-induced PC is abolished by HC, but only high-dose restored the cardioprotection	Fasudil up-regulated the PI3K/Akt/eNOS pathway and induced the opening of the mito-KATP channel	Wu et al., 2014b
NO donors induced late PC in rabbits	Hypercholesterolaemia blunted NO donor (diethylenetriamine/NO)-induced late PC	Disruption of biochemical pathways distal to the generation of NO	Tang et al., 2004

indicating that inhibition of nitrosative stress plays a role in cardioprotection (Andreadou *et al.*, 2012). These controversial results concerning the mechanisms of cardioprotection vary according to quality, composition and time of administration of the high-cholesterol diet, as well as the species used in each experiment.

Apoptosis and reperfusion injury salvage kinase signalling. Other factors that have been proposed to account for the larger myocardial infarction observed in hypercholesterolaemia include heat shock protein-70, which is down-regulated in hypercholesterolaemia (Csont *et al.*, 2002) and caspase-3, the activation of which is increased in hypercholesterolaemic ischaemic rabbit myocardium (Wang *et al.*, 2002). Hypercholesterolaemia prevents the sevoflurane-induced cardioprotection against

ischaemia/reperfusion injury by altering the upstream signalling of GSK3β (Xu *et al.*, 2013). In another study that investigated the cardioprotection of PostC in hypercholesterolaemic rat isolated hearts, it was observed that infarct size and cardiomyocyte apoptosis were completely abolished by hypercholesterolaemia due to the impairment of phosphorylation of GSK3β and attenuation of mitochondrial permeability transition pore (mPTP) opening (Wu *et al.*, 2014a).

The roles of reperfusion injury salvage kinases (RISK) and apoptosis-related pathways in the attenuation of cardioprotection of PostC were recently investigated in rat isolated hearts. The results showed that PostC significantly decreased the infarct size and apoptosis, and improved the functional recovery of ischaemic myocardium, but these beneficial effects were reversed by a high-cholesterol diet.

Moreover, hypercholesterolaemia inhibited the phosphorylation of Akt and ERK1/2, which were activated by PostC in normal hearts, and induced excessive apoptosis by down-regulating B-cell lymphoma 2 (Bcl-2) and up-regulating bcl-2-like protein 4, cytochrome c, caspase 9 and caspase 3. These results indicate that the hypercholesterolaemia-induced loss of cardioprotection conferred by PostC is associated with inactivation of the RISK signalling pathway and dysregulation of the downstream apoptosis-related pathway (Wu *et al.*, 2015a).

Furthermore, it has been shown that PostC reduces the myocardial injury in hypercholesterolaemic rats probably by the up-regulation of hypoxia-inducible factor 1-α (HIF-1α), which may be involved in the PostC-mediated cardioprotective mechanisms (Zhao *et al.*, 2009). This was further confirmed by another study, which showed that when dimethyloxalylglycine was given before PostC to up-regulate HIF-1α protein level, the degree of ischaemia/reperfusion injury was attenuated in hypercholesterolaemic rats suggesting that an up-regulation of HIF-1α may be one of the cardioprotective mechanisms of PostC against ischaemia/reperfusion injury in hypercholesterolaemia (Li *et al.*, 2014).

ATP-sensitive potassium channels. Divergent results exist on the role of both mitochondrial and sarcolemal ATP-sensitive potassium channels (K_{ATP} channels also known as $K_{ir}6.2$ channels) in hypercholesterolaemia. In rabbit isolated hearts, PostC reduced the infarct size in hypercholesterolaemic animals through the activation of adenosine A_1 receptors and K_{ATP} channels (Donato *et al.*, 2007). In contrast, the infarct-size reducing effect of either the nonselective K_{ATP} activator cromakalim or the selective mitoK_{ATP} activator diazoxide was lost in hearts of hyperlcholesterolaemic rats, showing that hypercholesterolaemia may influence K_{ATP} channel function in the heart. Although the mechanism by which hypercholesterolaemia inhibits the cardioprotective effect of K_{ATP} modulators is not known, altered energy metabolism as well as increased oxidative stress, but not changes in the expression levels of functional K_{ATP} protein expression in the heart, due to the cholesterol diet have been shown to be involved (Csonka *et al.*, 2014).

Mitochonrial permeability transition pore. The blocking of the mPTP with cyclosporine A (CsA) was investigated in order to determine whether it can restore the cardioprotection of PostC in hypercholesterolaemic rat hearts. It was concluded that the effect of PostC blocked by hypercholesterolaemia may be due to the excessive opening of the mPTP, and thus, inhibiting the mPTP with CsA is able to reverse this loss of cardioprotection observed during hypercholesterolaemia (Wu *et al.*, 2015c).

The effects of PostC in ischaemia/reperfusion injury in the presence of hypercholesterolaemia and the proposed mechanisms are summarized in Table 2.

Effect of hypercholesterolaemia on cardioprotection induced by remote conditioning

To the best of our knowledge, there is only one very recent study investigating whether remote preconditioning (RIPC)-induced cardioprotection is intact in hypercholesterolaemia. In this study, RIPC failed to reduce myocardial necrosis and apoptosis in hypercholesterolaemic myocardium in rats. Importantly, the authors found that inhibition of GSK3β reduced myocardial infarct size in hypercholesterolaemic hearts, but no additional cardioprotective effect was achieved when combined with RIPC, suggesting that acute GSK3β inhibition may provide a novel therapeutic strategy for hypercholesterolaemic patients during AMI, whereas RIPC is less effective due to signalling events that adversely affect GSK3β (Ma *et al.*, 2016).

Effect of hypercholesterolaemia on drug-induced cardioprotection

Although a number of drugs have been shown to be effective in preventing myocardial ischaemic-reperfusion injury, few are capable of preserving cardioprotection in the presence of hypercholesterolaemia (Balakumar and Babbar 2012). In addition to studies that refer to the application of PC as a manoeuvre, some studies have also examined the role of pharmacologically- induced PC during hypercholesterolaemia. It is well established that volatile anaesthetic-induced PC confers myocardial protection against ischaemia–reperfusion; however, hypercholesterolaemia abolished the sevoflurane-induced cardioprotection in rats (Ma *et al.*, 2013). Although sevoflurane-induced PC exerts delayed cardioprotection in normocholesterolaemic rats, this beneficial effect was blocked by hypercholesterolaemia probably by an effect on the iNOS/mitochondrial ATP-dependent K^+ channel pathway (Zhang *et al.*, 2012).

Fasudil, a Rho-kinase inhibitor, has been shown to induce pharmacological PC in rats. However, low-dose fasudil-induced PC is abolished by hypercholesterolaemia and only a high-dose restored the cardioprotection (Wu *et al.*, 2014b). Conversely, fasudil was effective at restoring the cardioprotection of PostC in the hypercholesterolaemic rat heart. This effect was mediated by the activation of the PI3K/Akt/eNOS signalling pathway and an increase in the myocardial NO content (Wu *et al.*, 2014c).

Another example of the effect of hypercholesterolaemia on drug-induced PC are NO donors, which have been shown to confer late PC against myocardial ischaemia/reperfusion in healthy rabbits. Hypercholesterolaemia blunted the late PC mediated by the NO donor (diethylenetriamine), indicating that the inhibitory effects of hypercholesterolaemia on NO donor-induced late PC in conscious rabbits are caused by the disruption of biochemical pathways distal to the generation of NO that triggers these adaptations (Tang *et al.*, 2004). In hyperlcholesterolaemic rat hearts, the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP), brain natriuretic peptide (BNP-32) and exogenous cGMP failed to induce cardioprotection, suggesting that the defect in cytoprotective signalling in the hypercholesterolaemic myocardium may reside downstream of cGMP elevation probably at the level of PKG (Giricz *et al.*, 2009).

The loss of pacing-induced PC could be recaptured by the key polyprenyl product farnesol in hypercholesterolaemia; however, farnesol-treatment did not influence cardiac NO content in the cholesterol-fed rats or in the normal fed rats (Ferdinandy *et al.*, 1998b). Furthermore, the infarct-size

Table 2

Effect of HC on cardioprotection induced by PostC

Experimental model	Effect of PostC	Proposed mechanism(s)	Reference
Isolated rat hearts subjected to PostC	Elimination of infarct size reduction and cardiomyocyte apoptosis	Impairment of phosphorylation of GSK3β and attenuation of mPTP opening	Wu et al., 2014a
Isolated rat hearts subjected to PostC	Elimination of infarct size reduction and apoptosis	Inactivation of RISK signal pathway and dysregulation of downstream apoptosis-related pathway	Wu et al., 2015a
Rats subjected to PostC	Elimination of infarct size limiting effects of PostC	HC blocked the cardioprotective effect of PostC at least in part via deterioration of the PostC-induced early increase in peroxynitrite formation	Kupai et al., 2009
Rats subjected to PostC	Myocardial infarction reduction was not attenuated by hypercholesterolemia	HIF-1α up-regulation	Zhao et al. 2009; Li et al., 2014
Rats subjected to PostC	Elimination of PostC cardioprotection	Elevation of ADMA	Landim et al., 2013
Isolated rabbit hearts subjected to PostC	Myocardial infarction reduction was not attenuated by hypercholesterolaemia	Activation of A_1 receptors and K_{ATP} channels	Donato et al., 2007
Rabbits subjected to PostC triggered by two different algorithms (six cycles of 10 s ischaemia separated by 10 s reperfusion and four cycles of 30 s ischaemia separated by 30 s reperfusion immediately after the end of the index ischaemia)	Elimination of infarct size limiting effects of PostC	Observational study	Iliodromitis et al., 2006
Rabbits subjected to PostC	Elimination of PostC cardioprotection	Increased oxidative and nitrosative stress	Iliodromitis et al., 2010
Rabbits subjected to PostC	Elimination of PostC cardioprotection	Decreased phosphorylation of eNOS	Andreadou et al., 2012
Mini swines subjected to PostC	Elimination of the reduction of the no-reflow and necrosis areas	Hypercholesterolaemia increased the area of no-reflow	Zhao et al., 2007
Sevoflurane-induced PostC in rats	Elimination of sevoflurane-induced cardioprotection	Alteration of upstream signalling of GSK3β	Xu et al., 2013

limiting effect of cromakalim (a nonselective K_{ATP} channel activator) or diazoxide (a selective mitoK_{ATP} channel activator) was lost in hypercholesterolaemic rats (Csonka et al., 2014).

It is well established that hypercholesterolaemia is accompanied by a decrease in cardiac NO content and increased nitro-oxidative stress; however, the role of NO donors in ischaemia/reperfusion injury with or without hypercholesterolaemia is not well established. Although NO treatment prior to or during the early reperfusion period can limit infarct size in preclinical studies, the excessive production of NO at the beginning of reperfusion reacts with ROS and forms peroxynitrite (see for reviews: Andreadou et al., 2015; Bice et al., 2016). The three clinical studies with NO donors that have been performed so far have revealed no evidence of infarct size reduction in patients treated with NO donors immediately prior to reperfusion. Additionally, high concentrations of NO can promote cellular injury, a situation that is possible in patients being treated with several co-medications

including nitrates. Hence, cardioprotection by NO donors should be demonstrated in experimental models with co-morbidities and relevant co-medications prior to clinical translation (Ferdinandy et al., 2014; Andreadou et al., 2015; Bell et al., 2016; Bice et al., 2016).

Effect of antihyperlipidaemic drugs on the ischaemic heart and cardioprotection

Statins. Elevated cholesterol levels can be decreased by diet, exercise and appropriate medical therapy. Among the various hypolipidaemic agents, statins confer the main benefit in treated patients by substantially decreasing cardiovascular morbidity and mortality. Apart from the significant decrease in cholesterol levels, statins also have pleiotropic effects, which, albeit exaggerated, may provide additional benefits. In fact, experimental and clinical studies have shown that statins are involved in a reduction in reperfusion injury and inflammatory reactions and in the improvement in the

microcirculation. Statins are implicated in the generation of intracellular mediators, which may prevent mPTP opening and therefore preserve mitochondrial integrity and the survival of cells (Ludman *et al.*, 2009; Antoniades and Channon, 2014; Mihos *et al.*, 2014). However, a growing body of evidence suggests that the cardioprotective potential of statins, associated with their pleiotropic and anti-inflammatory effects, is mediated by the up-regulation and activation of PPARα (Balakumar and Mahadevan 2012; Ravingerova *et al.*, 2015). Many experimental studies have shown that statins reduce infarct size in hypercholesterolaemic animals (Penumathsa *et al.*, 2007); therefore, in the present review, we will focus on the role of statin administration on the cardioprotective mechanisms (PC and PostC).

There are biological differences between lipophilic and hydrophilic statins. Lovastatin prevents the cardioprotective effect of PC when applied acutely but not when given chronically. The cardioprotective effect of PostC was attenuated when chronic lovastatin treatment was applied, whereas acute lovastatin treatment had no effect (Kocsis *et al.*, 2008). Furthermore, acute and chronic lovastatin treatment show differential effects on the p42/p44 MAPK pathway; only acute lovastatin treatment significantly increased p42/p44 MAPK phosphorylation. These effects of lovastatin might play a role in its differential action on cardioprotective mechanisms (Kocsis *et al.*, 2008) The most hydrophilic statin, pravastatin, at a dose in which serum cholesterol was not normalized, restored the infarct size-limiting effect of PC in hypercholesterolaemic rabbits, although it did not reduce the infarct size when it was administered without PC (Ueda *et al.*, 1999). The activation of ecto-5′-nucleotidase was suggested as a possible mechanism for the hypercholesterolaemia-induced retardation and pravastatin-mediated restoration of the cardioprotective effect of PC (Ueda *et al.*, 1999).

The loss of PostC benefits could be reversed by a 3 week simvastatin treatment, which limits the infarct size both in normo- and in hypercholesterolaemic rabbits subjected to ischaemia–reperfusion irrespective of the presence of PostC, while PostC is effective only in normocholesterolaemic animals. One should deduce that simvastatin also reduced total cholesterol and LDL plasma levels and attenuated the oxidative and nitrosative stress in the ischaemic myocardium (Iliodromitis *et al.*, 2010). However, the infarct size limitation by simvastatin was lost in hyperlcholesterolaemic animals, when simvastatin was administered for a short time period and did not possess hypolipidaemic activities (Andreadou *et al.*, 2012). In contrast, short-term administration of pravastatin at the same dose as simvastatin reduced infarction in cholesterol-fed rabbits independently of any lipid lowering effect, potentially through eNOS activation and the attenuation of nitro-oxidative stress. The open lactone ring chemical structure of pravastatin prevents its plasma protein binding by 100 times compared to simvastatin. This may partly be related to the high 45% unbound fraction of pravastatin sodium in plasma, which may interact actively with the endothelium and activate the eNOS/Akt signalling cascade (Andreadou *et al.*, 2012).

Fibrates. Fibrates, such as fenofibrate, bezafibrate, ciprofibrate and clofibrate, are PPARα agonists widely used clinically for treating dyslipidaemias (Staels *et al.*, 1998).

Fibrates have been suggested to improve the prognosis for ischaemic heart disease due to other non-lipid effects that are directly associated with the activation of PPARα, resulting in numerous changes in gene transcription including the genes regulating lipid metabolism (Schoonjans *et al.*, 1996; Ravingerova *et al.*, 2015). Although accumulating data have demonstrated the role of PPAR activation in mediating cardioprotection in the setting of ischaemia/reperfusion in various experimental animal models (Ravingerová *et al.*, 2012; Barlaka *et al.* 2013; Barlaka *et al.*, 2016), evidence for the effectiveness of fibrates in restoring the lost cardioprotection of PC or PostC in hyperlcholesterolaemic animals is scarce. Treatment with fenofibrate markedly restored the cardioprotective and infarct size limiting properties of PC in hypercholesterolaemic rat hearts, whereas it did not affect the cardioprotection by PC in normal rat hearts (Singh *et al.*, 2014). Although this effect has not yet been corroborated in subsequent studies and the underlying mechanism is unclear, it is pausible that activation of PPARα, which is markedly down-regulated in hypercholesterolaemic rat heart after ischaemia/reperfusion, and subsequent activation of the PI3K/Akt/eNOS pathway may restore the lost cardioprotection in hypercholesterolaemic hearts. In this context, PPARα up-regulation confers preconditioning-like protection against ischaemia/reperfusion via metabolic effects whereas PI3K/Akt activation may also be involved in the downstream mechanisms (Ravingerová *et al.*, 2012; 2015).

Niacin. Another hypolipidaemic drug with pleiotropic properties is nicotinic acid (niacin), which inhibits platelet activation, and reduces the expression of pro-inflammatory vascular cell adhesion molecule-1 (VCAM-1; Stach *et al.*, 2012) and oxidative stress (Gouni-Berthold and Berthold 2013), in addition to modulating the lipid profile. To the best of our knowledge, there are no data associating niacin with the cardioprotective effect of PC or PostC or its loss in the hypercholesterolaemic heart.

PCSK9 inhibitors. Inhibition of the proprotein convertase subtilisin-kexin type 9 (PCSK9) leads to an increased density of cell surface LDL receptors and therefore a reduction in serum LDL. Several monoclonal antibodies targeting PCSK9 have been developed recently and used as anti-hypercholesterolaemic drugs (Cohen *et al.*, 2006). Nevertheless, there are no data in the literature regarding the possible influence of PCSK9 inhibition on the cardioprotective effect of conditioning. Therefore, it would be of great importance to evaluate if these new anti-hypercholesterolaemic drugs can affect the efficacy or safety of cardioprotection elicited by conditioning strategies.

Conclusions and perspectives

Hypercholesterolaemia changes cardiac transcriptomics, cellular signalling and metabolism leading to mild diastolic dysfunction and endothelial dysfunction. Moreover, hypercholesterolaemia worsens the outcome of ischaemia/reperfusion injury and attenuates the cardioprotective effect of preconditioning, PostC, remote conditioning, as well as pharmacological

cardioprotection by interfereing with cardioprotective signalling pathways (Figure 1). Our review highlights the relative lack of experimental and especially clinical data looking at cardioprotection in treated or untreated hypercholesterolaemic animal models and patients, as well as the lack of knowledge on the effect of antihyperlipidaemic drugs on the ischaemic heart and cardioprotective signalling. Therefore, the establishment of more clinically relevant preclinical models of hypercholesterolaemia, together with the identification of novel targets are needed for the development of new cardioprotective drugs

Figure 1

Effect of hypercholesterolaemia on major known cardioprotective cellular mechanisms induced by conditioning interventions: Hypercholesterolaemia inhibits the phosphorylation of Akt and impairs the myocardial NO-cGMP pathway leading to inhibition of mitochondrial K_{ATP} channel opening. Additionally, impairment of the inhibition of GSK3β may cause excessive opening of the mPTP leading to mitochondrial swelling and cell death. Hypercholesterolaemia also inhibits the phosphorylation of extracellular-ERK1/2 and induces down-regulation of HIF-1α, which is one of the cardioprotective mechanisms of PostC. Hypercholesterolaemia produces excessive apoptosis by down-regulating Bcl-2 and up-regulating Bcl-2-like protein 4 (Bax), cytochrome c, caspase 9 and caspase 3. Hypercholesterolaemia inhibits mitochondrial translocation of connexin 43 (Cx43). Hypercholesterolaemia produces an increased generation of superoxide anion and a decreased bioavailability of NO through, for example, eNOS and iNOS uncoupling by a reduction in the NOS cofactor BH_4. Therefore, during hypercholesterolaemia, an increased formation of peroxynitrite, a toxic reaction product of superoxide and NO, is observed that further depletes bioavailability of NO in the heart. Moreover, the inhibition of oxidative activation of MMP2 by conditioning is blocked in hypercholesterolaemia due to peroxynitrite-induced activation of MMP2. Green boxes and dashed arrows denote major cardioprotective pathways that are affected by hypercholesterolaemia. Orange boxes and arrows indicate major influence of hypercholesterolaemia on cardioprotective cellular pathways.

that will be able to reverse the increased susceptibility of hypercholesterolaemic hearts to ischaemia/reperfusion injury and to provide cardioprotection.

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Conflict of interest

The authors declare no conflicts of interest.

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