Sphingosine-1-phosphate signals the way for Cx43-mediated cardioprotection

Richard D. Veenstra*

Department of Pharmacology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, New York 13210, USA

Online publish-ahead-of-print 2 November 2011

This editorial refers to ‘The natural cardioprotective particle HDL modulates connexin43 gap junction channels’ by S. Morel et al., pp. 41–49, this issue.

The onset of coronary occlusion caused by vasospasm, thrombosis, or atherosclerotic plaque rupture produces myocardial ischaemia, and acute myocardial infarction (AMI) can result within minutes. Reperfusion with minimal time lapse is the treatment of choice for AMI, but coronary reperfusion can also enhance myocardial injury and the induction of life-threatening cardiac arrhythmias. In 1986, it was fortuitously discovered that brief bouts of myocardial ischaemia prior to the onset of prolonged ischaemia and infarction could protect the myocardium and reduce infarct size.1 Cardioprotection—the promotion of cell survival, myocardial function, and reduction of infarct size—is conferred by brief episodes of ischaemia prior to prolonged occlusion, a phenomenon called ‘ischaemic preconditioning’ (IPC).1,2 High-density lipoproteins (HDL), containing the so-called ‘good cholesterol’, are known to induce IPC independent of their lipid-lowering effects.3 Connexin43 (Cx43), the major myocardial gap junction protein responsible for rapid, synchronous transmission of the cardiac action potential, was also obligatorily linked to IPC when it was observed that the heterozygous deletion of the Gja1 gene in (Cx43+/−) mice eliminated the response.4 This requirement for the presence of Cx43 for IPC to occur was subsequently linked to the preservation of Cx43 phosphorylation during prolonged ischaemia.5 However, a link between the cardioprotective effects of HDL and Cx43 has not previously been established.

An original study by Morel et al.6 establishes a causal link between the bioactive phospholipid, sphingosine-1-phosphate (S1P), an obligatory constituent of HDL hypothesized to mediate the direct cardioprotective effects of HDL,3–9 and the HDL-dependent phosphorylation of Cx43 by protein kinase C (PKC). In this study, a 5 min exposure of neonatal rat cardiomyocytes to HDL or S1P induced Cx43 phosphorylation at serine 368, a known Cx43 PKC phosphorylation site. These exposures reduced conduction velocity only slightly while significantly diminishing intercellular Lucifer Yellow dye transfer. These effects of HDL or S1P on Cx43 phosphorylation and fluorescent dye transfer were antagonized by pharmacological PKC inhibition, but the HDL-mediated phosphorylation of Cx43 was not blocked by p38/ERK/JNK-MAPK or Akt inhibitors. HDL or S1P exposure prior to 30 min of no-flow ischaemia in Langendorff rat heart preparations reduced infarct size by ~50%, consistent with previous findings.3–9 These results demonstrate that short-term HDL or S1P exposure modulates Cx43 gap junction function by a PKC-dependent mechanism known to produce IPC.

PKC plays a vital role in cardioprotection since PKC inhibition prevents IPC.10 There are numerous isoforms of PKC and two, PKC-δ and PKC-ε, have been directly linked to IPC in disparate ways. Activation of PKC-δ during ischaemia/reperfusion increases myocardial injury, although PKC-δ deletion also enhances ischaemic injury, suggesting that PKC-δ activation prior to ischaemia promotes cardioprotection. Conversely, PKC-ε activation during ischaemia/reperfusion invokes IPC and is a requirement for cardioprotection. Current ischaemia/reperfusion therapies favour administering PKC-ε activator (IκBαPKC) or PKC-δ inhibitor (δV1-1) peptides at the time of reperfusion to minimize myocardial injury and preserve function, although the two actions are not necessarily synergistic.2 PKC has many intracellular targets, and it is not fully understood which substrates are essential to IPC. Suggested targets include sphingosine kinases 1 and 2 (SK1&2), the mitochondrial permeability transition pore (mPTP), mitochondrial ATP-sensitive K channel (mKATP), BAX/BAD, Bcl-2, and Cx43.8–11

Cx43 phosphorylation at sites S262 and S368 is enhanced by IPC.10 IPC-inducing factors like fibroblast growth factor-2 (FGF-2) and diazoxide, an mKATP channel opener.11 Mutation of S262 to the phosphorylation-resistant alanine (S262A) prevented the pro-survival effects of PKC-ε-mediated Cx43 phosphorylation, although effects of S368 cannot be excluded since this site is also phosphorylated during IPC.12 Despite this evidence of PKC-ε-dependent phosphorylation of Cx43 being required for IPC, the precise mechanism of Cx43 cardioprotection remains poorly understood. Phosphorylation of Cx43 at S368 reduces gap junction-mediated electrical channel conductance and fluorescence dye transfer, the latter thought to reduce the spread of chemical pro-necrotic or pro-apoptotic factors and, thus, limit the infarct size.12

Cx43 is also proposed to mediate IPC by gap junction-independent mechanisms. Cx43 and PKC-ε are expressed in the mitochondria, and...
Cx43 hemichannels in the inner mitochondrial membrane (IMM) apparently contribute to mitochondrial K⁺ influx necessary for reactive oxygen species (ROS)-dependent activation of IPC. Mitochondrial Cx43 content is predominantly phosphorylated and is increased by IPC. Despite these observations, the precise mechanism by which Cx43, and its phosphorylation, produces IPC remains unknown. The work by Morel et al. provides evidence for an HDL/S1P-dependent PKC signalling cascade targeting Cx43, summarized in Figure 1, that somehow operates independently of the PI3K/Akt pathway. The readout for PKC-mediated Cx43 phosphorylation was the appearance of phospho-S368, which was not necessarily linked to IPC in this study. The only data that directly address IPC in the current study is the reduction in infarct size by HDL or S1P perfusion in Langendorff-perfused hearts. The effect of MAPK, Akt, and PKC inhibitors on Cx43 pS368 content was only performed in the presence of HDL. Previous studies have indicated that the cardioprotective effects of S1P are mediated via the S1P3 receptor and a PI3K/Akt NO-dependent pathway. Data from the Morel et al. study suggest that PKC-mediated phosphorylation of Cx43 is induced by HDL even when PI3K is inhibited by LY294002. The present study does not discern between PKC isoforms since the PKC inhibitors used will effectively inhibit both PKC-δ and PKC-ε. The S1P signalling pathway should be explored in future experiments to determine the possible role of S1P receptors, PKC-δ, and PKC-ε using specific activators or inhibitors (e.g. FTY720, VPC23019, SEW2871, 8V1-1, 14βRACK) in the HDL/S1P-mediated phosphorylation of Cx43. Phosphorylation of Cx43 at S262 and S368 should be specifically considered as well as the cellular localization of Cx43 to gap junctions or the IMM. Even with this information, the precise mechanistic basis for Cx43-induced cardioprotection will require functional studies of Cx43 channels and possible protein scaffolding interactions. The HDL/S1P signalling pathway provides a mechanistic basis to possibly explain the non-lipid-lowering effects of HDL on Cx43 and their respective roles in cardioprotection.

Conflict of interest: none declared.

References