TRPing up reperfusion: neutrophil TRPM2 channels exacerbate necrosis and contractile dysfunction in post-ischaemic myocardium

Ronald J. Korthuis* and Theodore Kalogeris

Department of Medical Pharmacology and Physiology and the Dalton Cardiovascular Research Center, University of Missouri School of Medicine, One Hospital Drive, Columbia, MO 65212, USA

Online publish-ahead-of-print 11 December 2012

This editorial refers to ‘Neutrophil TRPM2 channels are implicated in the exacerbation of myocardial ischaemia/reperfusion injury’ by T. Hiroi et al., pp. 271–281, this issue.

Myocardial infarction, stroke, and other ischaemic disorders are leading causes of morbidity and mortality. As a consequence, intensive research efforts have been directed at determining the mechanisms underlying cell death and dysfunction induced by ischaemia. Although reperfusion is essential to rescue ischaemic tissues, re-establishing the blood supply is not without peril as it can provoke overproduction of reactive oxygen and nitrogen species (ROS, RNS), inflammation, and an excessive rise in intracellular calcium levels as common triggers for cell dysfunction and death in post-ischaemic tissues. Thus, oxidative/nitrosative stress, neutrophils, other immunocytes, and calcium overload are now recognized as key contributors to the reperfusion-induced component of total tissue injury sustained in organs subjected to ischaemia/reperfusion (I/R).1

Although a number of calcium channels have been implicated in I/R, recent work has focused attention on the potential role of redox-sensitive transient receptor potential melastatin 2 (TRPM2) channels as novel mediators.2–5 These channels consist of six transmembrane domains and intracellular N and C termini that form monovalent cation channels with selectivity for Ca2+ and to a lesser extent Mg2+ 2–5. TRPM2 channels are expressed by a variety of cells and together with TRPM6 and TRPM7 are unique among ion channels in that their C-terminus contains enzymatic domains that exhibit ADP ribose (ADPR) pyrophosphatase activity, providing this domain with a site for ADPR binding and activation of the channel.2–5 With regard to mechanisms relevant to I/R, TRPM2 channels are also activated by direct oxidation of channel moieties by H2O2 or indirectly via activation of enzymatic pathways [poly(ADPR) polymerase (PARP), poly(ADPR) glycohydrolase, or the NAD/CADPR glycohydrolases CD38 and CD157] involved in the formation of ADPR.2–5 On the basis of this information, it is tempting to speculate that the anti-inflammatory and infarct-sparing effects of PARP inhibition in cardiac I/R may be related to attendant reductions in TRPM2 activation. This mechanism is also thought to mediate TRPM2 activation by tumour necrosis factor-α (TNF-α), an inflammatory mediator that plays a key role in the pathogenesis of I/R.5,6 Changes in intracellular Ca2+ levels also facilitate channel activation by ADPR,2–5 which further supports a potential role for TRPM2 in the pathogenesis of I/R. Indeed, knockdown of TRPM2 expression or pharmacological inhibition of channel activity has been shown to reduce neural injury in experimental stroke.8,9

In this issue of Cardiovascular Research, Hiroi et al.10 not only extend these observations to myocardial I/R injury, they further show that TRPM2 channels expressed on neutrophils play a key role in exacerbating post-ischaemic contractile function and the size of an evolving infarct, but do not contribute to the ischaemic component of total tissue injury in I/R (Figure 1). The latter observation is consistent with the effect of ischaemia to induce acidosis, which has been shown to inhibit TRPM2.4 The most convincing evidence provided by Hiroi et al.10 to support this novel role for neutrophil TRPM2 in myocardial reperfusion injury is derived from studies showing that: (i) myocardial infarct size and contractile dysfunction were reduced after I/R, but not ischaemia alone, in TRPM2 knockout mice (TRPM2+/−) compared with wild-type animals (WT); (ii) cardiac neutrophil infiltration was reduced after I/R in TRPM2+/− vs. WT mice; (iii) an increased intracellular Ca2+ concentration and increased endothelial cell adhesive interactions were noted for neutrophils obtained from WT but not TRPM2+/− mice exposed to H2O2 plus leukotriene B4 (LTB4), inflammatory mediators that have been implicated in post-ischaemic leukoestration;1 and (iv) infusion of TRPM2+/− neutrophils into ex vivo perfused WT hearts limited I/R injury, whereas an infusion of WT neutrophils into TRPM2-deficient hearts enlarged infarct size. To rule down the possibility of compensatory changes in gene expression induced by TRPM2 knockout, acute pharmacological inhibition of the channel in WT animals also reduced infarct size.

Although the aforementioned results clearly support the notion that neutrophil TRPM2 is involved in exacerbating reperfusion injury in the heart, some of the findings were not consistent with this conclusion. For example, only very modest differences in Mac-1 expression (an adhesion molecule mediating firm adhesion induced by I/R) were noted for neutrophils obtained from WT vs.
The formation of NADPH oxidase-derived ROS by activated neutrophils is a major contributor to oxidative stress in I/R. However, recent work indicates that lipopolysaccharide (LPS)-induced, NADPH oxidase-mediated ROS production is enhanced in TRPM2−/− neutrophils. While apparently difficult to reconcile with the infarct-sparing actions of neutrophil TRPM2 deficiency reported in the study by Hiroi et al., it is possible that the inhibitory effect on TRPM2-dependent neutrophil oxidant production is stimulus dependent, since LPS plays a more important role in sepsis and not in myocardial I/R injury (unless severe enough to compromise intestinal perfusion, which can lead to bacterial translocation to abluminal compartments and lymph). Hiroi et al. also noted that myocardial injury was not significantly increased by reperfusion with perfusate devoid of neutrophils, which is inconsistent with numerous other reports in the literature. This discrepancy probably relates to the model of I/R used or perhaps species/mouse strain differences.

A question that requires resolution in future studies relates to the fact that young male mice were used in all studies reported by Hiroi et al. Although these results are an informative first step, it is not clear whether the results extrapolate to females (Figure 1). This is an important consideration given recent reports indicating that there are sex differences in neuroprotection provided by TRPM2 inhibition in experimental stroke, which is effective in male but not female animals. It is also not clear whether the exacerbating effect of neutrophil TRPM2 activation to increase infarct size and reduce contractile function after I/R will manifest to the same extent in the presence of other confounding risk factors such as advancing age, diabetes, hypertension, obesity, and/or dyslipidaemias (Figure 1). Finally, TRPM2 channels are also expressed on fibroblasts, lymphocytes, monocytes/macrophages, and dendritic cells, which contribute to the pathogenesis of I/R. It will be very interesting to determine whether the absence of TRPM2 on these cells also limits myocardial I/R (Figure 1).}

TRPM2−/− mice after exposure to H2O2 and LTB4. Moreover, the enhanced migratory responses to H2O2 and LTB4 or increased intracellular Ca2+ induced by platelet-activating factor (PAF) or C5a plus H2O2 exhibited by WT and TRPM2−/− neutrophils were not different from each other. These disparate findings are most probably reconciled by the possibility that I/R induces the expression of multiple chemotactic factors that may act in concert to activate TRPM2 on neutrophils. In addition, there is evidence for differential activation of Ca2+ influx pathways in neutrophils, with PAF activating channels distinct from TRPM2. It is also possible that additional factors not present in this in vitro assay may be required to link communication with inflammatory mediator receptors and TRPM2 on neutrophils, as has been shown for soluble E-selectin, which permits cross-talk between PAF receptors and TRPC channels (neutrophils). Future studies should be directed at the evaluation of the effect of TNF-α alone or in combination with H2O2 on these indices of activation in WT and TRPM2−/− neutrophils because this cytokine has been shown to play a dominant role in post-ischaemic myocardial injury and is a well-documented activator of TRPM2.

In summary, the study by Hiroi et al. provides fascinating, novel, and important insights regarding the pathogenic role of neutrophil TRPM2 activation in the wave front of reperfusion injury. Like all well-designed and executed studies that report novel findings, this study raises as many intriguing questions worthy of future investigation (Figure 1) as it has answered.

Conflict of Interest: none declared.

Funding

The authors’ work is supported by grants from the US National Institutes of Health (HL-095486 and AA-014945).

References


Figure 1 The study by Hiroi et al. in this issue of Cardiovascular Research clearly establishes a role for TRPM2 channels expressed on neutrophils in the pathogenesis of reperfusion-induced myocardial dysfunction and cell death (red boxes). The work also raises a number of intriguing questions (Q) that require future investigation to address: (Q1) What factors activate TRPM2 channels on neutrophils during reperfusion? (Q2) Does post-ischaemic leukocyte rolling along, adherence to, and/or emigration across post-capillary venular endothelium occur by a TRPM2-dependent mechanism? (Q3) What intracellular signalling mechanisms couple reperfusion-induced TRPM2 channel activation on neutrophils with cardiac myocyte injury? (Q4) Does ischaemia-induced acidosis prevent neutrophil TRPM2 channel activation? (Q5) Does reperfusion activate other immune cells (e.g. monocytes, macrophages, lymphocytes, and/or dendritic cells) to contribute to tissue injury via production of mediators that promote the calcium influx into these cells via TRPM2 channels? (Q6 and Q7) Does TRPM2 activation on these other immune cells contribute to post-ischaemic tissue injury by elaboration of TNF-α or other mediators that activate neutrophil TRPM2 (Q6) or do they directly contribute to myocyte death by a TRPM2-dependent mechanism (Q7)? (Q6) Does advancing age, sex, or other risk factors influence TRPM2 activation on neutrophils during reperfusion of ischaemic tissues? ADPR, ADP ribose; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-α; PIM, pro-inflammatory mediators.

TRPM2−/− mice after exposure to H2O2 and LTB4. Moreover, the enhanced migratory responses to H2O2 and LTB4 or increased intracellular Ca2+ induced by platelet-activating factor (PAF) or C5a plus H2O2 exhibited by WT and TRPM2−/− neutrophils were not different from each other. These disparate findings are most probably reconciled by the possibility that I/R induces the expression of multiple chemotactic factors that may act in concert to activate TRPM2 on neutrophils. In addition, there is evidence for differential activation of Ca2+ influx pathways in neutrophils, with PAF activating channels distinct from TRPM2. It is also possible that additional factors not present in this in vitro assay may be required to link communication with inflammatory mediator receptors and TRPM2 on neutrophils, as has been shown for soluble E-selectin, which permits cross-talk between PAF receptors and TRPC channels (neutrophils). Future studies should be directed at the evaluation of the effect of TNF-α alone or in combination with H2O2 on these indices of activation in WT and TRPM2−/− neutrophils because this cytokine has been shown to play a dominant role in post-ischaemic myocardial injury and is a well-documented activator of TRPM2.

The formation of NADPH oxidase-derived ROS by activated neutrophils is a major contributor to oxidative stress in I/R. However, recent work indicates that lipopolysaccharide (LPS)-induced, NADPH oxidase-mediated ROS production is enhanced in TRPM2−/− neutrophils. While apparently difficult to reconcile with the infarct-sparing actions of neutrophil TRPM2 deficiency reported in the study by Hiroi et al., it is possible that the inhibitory effect on TRPM2-dependent neutrophil oxidant production is stimulus dependent, since LPS plays a more important role in sepsis and not in myocardial I/R injury (unless severe enough to compromise intestinal perfusion, which can lead to bacterial translocation to abluminal compartments and lymph). Hiroi et al. also noted that myocardial injury was not significantly increased by reperfusion with perfusate devoid of neutrophils, which is inconsistent with numerous other reports in the literature. This discrepancy probably relates to the model of I/R used or perhaps species/mouse strain differences.

A question that requires resolution in future studies relates to the fact that young male mice were used in all studies reported by Hiroi et al. Although these results are an informative first step, it is not clear whether the results extrapolate to females (Figure 1). This is an important consideration given recent reports indicating that there are sex differences in neuroprotection provided by TRPM2 inhibition in experimental stroke, which is effective in male but not female animals. It is also not clear whether the exacerbating effect of neutrophil TRPM2 activation to increase infarct size and reduce contractile function after I/R will manifest to the same extent in the presence of other confounding risk factors such as advancing age, diabetes, hypertension, obesity, and/or dyslipidaemias (Figure 1). Finally, TRPM2 channels are also expressed on fibroblasts, lymphocytes, monocytes/macrophages, and dendritic cells, which contribute to the pathogenesis of I/R. It will be very interesting to determine whether the absence of TRPM2 on these cells also limits myocardial I/R (Figure 1). Macrophages and dendritic cells are important sources of TNF-α and other cytokines and chemokines that may serve to amplify the inflammatory response to I/R, perhaps by a TRPM2-dependent mechanism. Indeed, TRPM2-mediated Ca2+ influx increases chemokine production by monocytes and macrophages, which promotes neutrophil infiltration.

In summary, the study by Hiroi et al. provides fascinating, novel, and important insights regarding the pathogenic role of neutrophil TRPM2 activation in the wave front of reperfusion injury. Like all well-designed and executed studies that report novel findings, this study raises as many intriguing questions worthy of future investigation (Figure 1) as it has answered.

Conflict of Interest: none declared.

Funding

The authors’ work is supported by grants from the US National Institutes of Health (HL-095486 and AA-014945).

References


