Targeting lysosomal Ca\(^{2+}\) to reduce reperfusion injury

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This editorial refers to ‘Inhibition of NAADP signalling on reperfusion protects the heart by preventing lethal calcium oscillations via two-pore channel 1 and opening of the mitochondrial permeability transition pore’ by S.M. Davidson et al., pp. 357–366.

The use of percutaneous coronary intervention (PCI) in patients with acute myocardial infarction (AMI) has successfully reduced the incidence of associated mortality and/or morbidity. However, despite widespread use of PCI, there has been an increase in the prevalence of ischaemic heart failure, which is mainly attributable to the fact that this treatment has not been as successful as expected in limiting the size of infarcts. Because reducing the infarct size is essential to decrease the development of heart failure, PCI alone is not sufficient to reduce the incidence of ischaemic heart failure following AMI. To overcome this challenge, many researchers have investigated the major causes of ischaemia and reperfusion injury and methods to attenuate the damage caused by them.\(^1\)

1. Cellular mechanisms underlying reperfusion injury

Many lines of evidence for the causes of ischaemia and reperfusion injury implicate mitochondrial dysfunction. Myocardial ATP depletion through mitochondrial dysfunction decreases Ca\(^{2+}\) uptake into the sarcoplasmic reticulum (SR) in ischaemic/reperfused myocardium, leading to an increase in intracellular Ca\(^{2+}\) [Ca\(^{2+}\)]\(_i\) levels. These findings suggest that the phenomena of ATP depletion and Ca\(^{2+}\) overload may play a central role in ischaemia and reperfusion injury; in contrast, Ca\(^{2+}\) is also important for maintaining normal cardiac performance, thus demonstrating that [Ca\(^{2+}\)]\(_i\) has both a physiological and a pathological role in the heart.

The levels of cyclic ADP-ribose (cADPR), one of the secondary messengers for Ca\(^{2+}\) signalling, are increased by Ca\(^{2+}\) influx across the plasma membrane. cADPR itself stimulates ryanodine receptors (RyRs) located on the SR,\(^3\) which contributes to an increase in [Ca\(^{2+}\)]\(_i\) levels in cardiomyocytes. Lysosomes, which act as scavengers by degrading impaired amino acids, lipids and proteins\(^3\) can also contribute to Ca\(^{2+}\) overload. Two-pore channels (TPCs) located on lysosomes are stimulated by the secondary messenger nicotinic acid adenine dinucleotide phosphate (NAADP) to stimulate Ca\(^{2+}\) release. Thus, many pathways lead to accumulation of [Ca\(^{2+}\)]\(_i\) via Ca\(^{2+}\) channels or Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers across the cytoplasmic membrane, and via Ca\(^{2+}\) release from the SR and lysosomes. Ca\(^{2+}\) overload induces Ca\(^{2+}\) oscillations, which may eventually lead to ventricular fibrillation. Both Ca\(^{2+}\) overload and Ca\(^{2+}\) oscillations were shown to open mitochondrial permeability transition pores (mPTPs), thereby causing cellular necrosis and apoptosis.\(^4\) Importantly, the relative role of [Ca\(^{2+}\)]\(_i\), via the different routes for either normal homeostasis or for the detrimental process of reperfusion injury has not been clearly established. In the present issue, a study by Davidson et al.\(^5\) investigated the contribution of TPCs and NAADP.

2. Novelty, strengths, and impact of the present work

Several important issues related to the cellular mechanisms underlying reperfusion injury remain unresolved. Most importantly, although many researchers have recognized that Ca\(^{2+}\) overload plays an important role in reperfusion injury, no study has demonstrated which organelles are responsible for Ca\(^{2+}\) overload and Ca\(^{2+}\) oscillation. The major pathways that cause Ca\(^{2+}\) overload in cardiomyocytes are believed to involve SR, lysosomes, or Ca\(^{2+}\) influx across the membrane via Na\(^+\)/Ca\(^{2+}\) exchangers.\(^6\) The present study by Davidson et al. demonstrates that NAADP signalling plays a major role in increases in [Ca\(^{2+}\)]\(_i\), and Ca\(^{2+}\) oscillation, and subsequent reperfusion-induced cell death via mPTP opening.\(^7\) They developed the novel compound Ned-K by replacing the fluoride of Ned-19 to a cyano group, which is a more soluble inhibitor of NAADP than Ned-19,\(^8\) and found that Ned-K inhibits the increases in [Ca\(^{2+}\)]\(_i\); and Ca\(^{2+}\) oscillation in cardiomyocytes during reoxygenation, resulting in the prevention of cellular death. Ned-K delayed mPTP opening; this was not due to a direct effect of Ned-K on the mPTPs, since Ca\(^{2+}\)-induced swelling of isolated mitochondria was unaffected by Ned-K. As Ned-19 inhibits RyRs in addition to lysosomes, Davidson et al. carefully tested whether this is also the case for Ned-K. They found

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that the spontaneous Ca$^{2+}$ increase via RyR activation, a specific SR function, was not inhibited by Ned-K. Furthermore, they provided evidence that treatment of cardiomyocytes with glycyl-l-phenylalanine-beta-naphthylamide, which permeabilizes lysosomes via osmotic lysis, inhibits Ca$^{2+}$ oscillation. These findings indicate that Ca$^{2+}$ overload, Ca$^{2+}$ oscillation, and mPTP opening may be largely attributed to Ca$^{2+}$ release via lysosomes. Finally, they found that Ned-K, but not Ned-19, decreases the infarct size following ischemia and reperfusion in mice. Moreover, they used mice lacking TPC1 and found that these mice were protected against cardiac ischemia and reperfusion.

Taken together, the most important and novel points of the present study are considered to be the following: (i) the authors clarified the involvement of NAADP signalling and thus the increase in [Ca$^{2+}$]$_c$ via lysosomes in the reperfusion injury of the heart and (ii) they developed Ned-K as a NAADP antagonist and showed Ned-K-mediated cardioprotection via inhibition of Ca$^{2+}$ overload, Ca$^{2+}$ oscillation and consequently mPTP opening, in a similar manner to cyclosporine. Based on the present findings, a diagram on how Ca$^{2+}$ overload occurs and causes cardiovascular injury can be proposed (Figure 1).

This study is particularly important for guiding future research to target lysosomes as a contributor to Ca$^{2+}$ homeostasis, a pathway that has been ignored for a long time because lysosomes are believed to play a role as scavengers of intracellular substances such as lipids, amino acids, and proteins. The endoplasmic reticulum (ER) and SR are also

![Figure 1](https://example.com/image.png)  
**Figure 1** Schematic illustration for Ca$^{2+}$ overload-induced injury of cardiomyocytes. The major pathways that cause Ca$^{2+}$ overload in cardiomyocytes involve ER/SR, lysosomes, or Ca$^{2+}$ influx across the membrane via Na$^+$/Ca$^{2+}$ exchangers. The culminated Ca$^{2+}$ overload via several pathways causes mitochondrial dysfunction including mPTP opening via Ca$^{2+}$ oscillation, and provokes necrosis and/or apoptosis of cardiomyocytes. The present study highlighted that NAADP signalling plays a major role in increases in [Ca$^{2+}$]$_c$, via lysosomes, and subsequent reperfusion-induced cell death via mPTP opening; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; IP3, 1,4,5-trisphosphate; IP3R, the receptor for IP3; RyR, ryanodine receptor; cADPR, cyclic ADP-ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; TPC, two-pore channels; ROS, reactive oxygen species; Mt, mitochondrion; mPTP, mitochondrial permeability transition pore.
3. Unresolved issues and future perspectives

The present work has some limitations and issues. First, the reason for the differences between the infarct size-limiting effects of Ned-19 and Ned-K was not examined. Since both Ned-19 and Ned-K attenuated the increases in $[\text{Ca}^{2+}]_c$ and Ca$^{2+}$ oscillation via lysosomes, both drugs should have limited infarct size. The authors discussed this complicated issue by a hypothetical idea that SR-mediated Ca$^{2+}$-handling, which can be inhibited by Ned-19 but not by Ned-K, is cardioprotective. Since myocardial contraction—relaxation is regulated by Ca$^{2+}$ via SR, the roles of Ca$^{2+}$ via either SR or lysosomes in cardioprotection may be different: the quantitative or qualitative differences in rise of $[\text{Ca}^{2+}]_c$ during one cardiac cycle may explain such differences.

Second, before bringing Ned-K to the clinic as a drug-targeting ischaemia and reperfusion injury, the safety issues associated with LED-K use remain to be explored. In addition, the effects of Ned-K on haemodynamic parameters, such as blood pressure and heart rate, need to be elucidated. Such measurements are necessary to determine whether the compound is safe for future clinical use as well as to know its effects on haemodynamic parameters that may alter the infarct size even in the absence of direct cardioprotective effects of Ned-K. Heart rate and systemic blood pressure are known to substantially change the infarct size.

Third, the authors should still clarify the non-specific effects of Ned-K because the chemical compound may have other effects that influence infarct size limitation.

Fourth, the interaction between lysosomes and SR for the regulation of $[\text{Ca}^{2+}]_c$ and Ca$^{2+}$ overload is still to be demonstrated and the quantitative contribution of lysosomes and SR to Ca$^{2+}$ overload should be clarified in reperfused hearts.

Finally, although cyclosporine blocks mPTP, cyclosporine did not limit the size of infarcts in a recently conducted large-scale human clinical trial, suggesting that mPTPs, possible end-effectors of Ned-K, may not be cardioprotective for patients with AMI. These issues should be seriously considered in future studies.

Nonetheless, the study is a major step forward in demonstrating the importance of lysosomes in Ca$^{2+}$ homeostasis and the potential of Ned-K as a drug targeting ischaemic heart diseases. Importantly, Davidson et al. demonstrate that targeting this pathway at the time of reperfusion (and not only in pre-treatment) can reduce perfusion injury, showing the potential for clinical application.

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