Repurposing of an antiasthmatic drug may reduce NETosis and myocardial ischaemia/reperfusion injury

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This editorial refers to ‘Myocardial reperfusion injury exacerbation due to ALDH2 deficiency is mediated by neutrophil extracellular traps and prevented by leukotriene C4 inhibition’, by K. Yang et al., https://doi.org/10.1093/eurheartj/ehae205.

Graphical Abstract

ALDH2 deficiency and LTC4 synthesis enhance NET formation during myocardial I/RI. Myocardial ischaemia results in down-regulation of mitochondrial ALDH2 in the heart. Aldehyde dehydrogenase-2 (ALDH2) deficiency promotes endoplasmic reticulum (ER) stress-mediated reactive oxygen species (ROS) production via microsomal glutathione-S-transferase 2 (MGST2)-based biosynthesis of leukotriene C4 (LTC4). ROS mediate myocardial reperfusion injury by inducing the opening of the mitochondrial permeability transition pore (MPTP), acting as a neutrophil chemoattractant, and mediating dysfunction of the sarcoplasmic reticulum. Factors such as ROS, protein-arginine deiminase type-4 (PAD4), interleukin-1β, and tumour necrosis factor-α induce the formation of neutrophil extracellular traps (NETs) by activated neutrophils. The lack of proper clearance by macrophages as well as defects in DNase enzymes contributes to the release of the NET-derived debris. The NET contents are exposed to the immune system and are identified as autoantigens, driving autoantibody production, increased inflammation, tissue damage, and pathological fibrosis. Inhibition of the LTC4 receptor abolishes ER stress-induced ROS production and NET formation, contributing to cardioprotective effects during myocardial ischaemia/reperfusion injury (I/RI). Repurposing the antiasthmatic drug pranlukast, an FDA-approved LTC4 receptor antagonist, may serve as potential therapeutic strategy for myocardial I/RI. MMP, matrix metalloproteinase; NOX2, NAPDH oxidase 2

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Myocardial ischaemia/reperfusion injury (I/RI) remains a formidable challenge in cardiovascular medicine, necessitating a deeper understanding of the molecular mechanisms at play. Myocardial I/RI pathophysiology involves complex interactions between oxidative stress, cell death, and inflammation.  

1. Myocardial ischaemia often occurs in individuals who exhibit symptoms of an acute ST-segment elevation myocardial infarction (STEMI).  

The most efficient therapeutic approach for diminishing acute myocardial ischaemic injury in these patients and mitigating the extent of myocardial infarction is prompt opening of the occluded coronary artery by primary percutaneous coronary intervention. Reperfusion strategies, while being the standard therapy for acute myocardial infarction, can result in various forms of IRI, including reperfusion-induced arrhythmias, myocardial stunning, microvascular obstruction, and thrombosis.  

1. Despite advances in these revascularization therapies, the extent of myocardial damage and adverse left ventricular remodelling, reflected by significant mortality (7% death at 1 year) and morbidity (22% prolonged or new hospitalization for heart failure at 1 year), compromise the survival and quality of life in STEMI patients.  

Therefore, understanding the pathophysiology of myocardial I/RI is critical in addressing the unmet need for novel therapeutic strategies to protect the heart and improve the clinical outcomes of these patients.

Neutrophils, the immune front-line cell population, are the first responders to infiltrate the ischaemic myocardium drawn towards cellular debris and inflammatory mediators released by activated resident cells.  

2. Their defence mechanisms encompass phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs).  

NETs are web-like structures composed of chromatin and granular proteins that are released by activated neutrophils to trap and kill pathogens in a process called NETosis.  

However, excessive or inappropriate NETosis can also cause inflammation and tissue damage in various pathological conditions, such as atherosclerosis, thrombosis, sepsis, and auto-immune diseases.  

3. Indeed, during myocardial I/RI, enhanced activation and priming of neutrophils at the site of injury by factors such as damage-associated molecular patterns, oxidative stress, and inflammation promote the polarization of neutrophils toward an inflammatory phenotype. This triggers the sustained release of NET-induced agents such as myeloperoxidase (MPO), neutrophil elastase, and reactive oxygen species (ROS), leading to excessive NETosis, which further augments cardiac injury.  

While multiple stimuli are involved in NET formation, mitochondria are a major driving force behind this process through the generation of mitochondrial ROS.  

4. Mitochondria are particularly vulnerable to I/RI. The impairment of mitochondrial mitochondrial oxidative phosphorylation during ischaemia increased ROS and reduced levels of ATP production.  

5. The subsequent influx of oxygen upon reperfusion exacerbates mitochondrial dysfunction, triggering a cascade of events, including oxidative stress, calcium overload, and endoplasmic reticulum (ER) stress, that further fuels the myocardial damage.  

In addition, the inhibition of the opening of mitochondrial permeability transition p d ischaemia and their subsequent opening upon reperfusion further triggers the release of ROS and proapoptotic factors that lead to cardiomyocyte death.  

6. Aldehyde dehydrogenase-2 (ALDH2), a key mitochondrial enzyme expressed in various tissues such as the heart, liver, and kidney, metabolizes toxic aldehydes, which are by-products of alcohol metabolism.  

7. ALDH2 rs671 (Glu504Lys) mutations, a highly prevalent polymorphism ranging from 28% to 45% in East Asian countries while nearly absent in other populations, is associated with an increased risk of coronary artery disease, myocardial infarction, and heart failure.  

These mutations do not affect ALDH2 protein expression, but are related to reduced activity of the ALDH2 enzyme. The enzyme activity of the heterozygous mutant (GA) is only 13%–14% of that of the wild-type genotype (GG), while the homozygous mutant (AA) has almost no enzyme activity.  

8. The role of ALDH2 is underlined by the inverse correlation between ALDH2 activity and cardioprotection against I/RI damage in pre-clinical models. While the cardioprotective benefits of ALDH2 are partly due to the restoration of mitochondrial function through reduction of ROS production and clearance of toxic aldehydes,  

9. the specific mechanisms that lead to worsened myocardial I/RI when ALDH2 is deficient remain unclear.

In this issue of the European Heart Journal, Yang et al. report a novel mechanism by which ALDH2 deficiency exacerbates myocardial I/RI by promoting NET formation.  

10. The authors first demonstrate that ALDH2 rs671 mutant neutrophils (GA and AA) are more prone to NETosis than those carrying the ALDH2 rs671 wild-type mutation (GG).  

11. Using a mouse model of myocardial I/RI, the authors demonstrated that ALDH2 deficiency enhances NETosis in the ischaemic myocardium via up-regulating the ER stress/microsomal glutathione S-transferase 2 (MGST2)/leukotriene C4 (LTC4)/NADPH oxidase pathways, leading to increased ROS production.  

12. The authors further demonstrated that pharmacological inhibition of NETosis in a mouse model of myocardial I/RI injury using NET-degrading enzymes, peptidylarginine deiminase 4 inhibitor (GSK484), or DNase I significantly attenuated myocardial I/RI in both wild-type and ALDH2-deficient mice.  

Interestingly, the authors elegantly demonstrated that pranlukast, a Food and Drug Administration (FDA)-approved LTC4 receptor antagonist, had a similar protective effect, suggesting a potential repurposing of this drug for the treatment of myocardial I/RI, especially in individuals with ALDH2 mutation.  

13. Since the repurposed drug has already demonstrated a safety profile in the clinic, this strategy entails a reduction in drug development and investment time.  

Finally, Yang et al. enrolled 250 STEMI patients and reported that higher serum levels of the NETosis markers MPO-DNA and LTC4 were associated with adverse left ventricular remodelling at 6 or 12 months after primary percutaneous coronary intervention, highlighting a potential role as predictive biomarkers.  

14. Validation of these biomarkers could help in the risk stratification and could potentially be used to identify high-risk patients who might benefit from more aggressive or targeted interventions. Collectively, this study demonstrates that ALDH2 deficiency exacerbates myocardial I/RI by promoting NETosis and identifies NETosis and the LTC4 receptor as potential therapeutic targets for myocardial I/RI (Graphical abstract).

Neutrophils migrate into the heart shortly after reperfusion, reaching their highest levels around 1 day post-I/RI and persisting for ~3–4 days, meaning that NETosis mainly occurred within this short period after myocardial I/RI. Increased neutrophil circulation has a direct positive correlation with the extent of myocardial infarction, mortality rate, and the onset of cardiac failure.  

Also, experimental studies suggest that strategies aimed at neutrophil depletion or inhibition can mitigate myocardial damage and decrease infarct size. Nonetheless, neutrophils are essential for organ repair through resolution of inflammation and clearance of cellular debris.  

15. Since pranlukast exerts an anti-inflammatory effect by reducing neutrophil accumulation after brain ischaemia, blocking neutrophil infiltration may be deleterious at the late phase of organ I/RI.  

Thus, it seems necessary to investigate whether the decreased neutrophil infiltration causes the decreased NET formation or whether the drug or treatment itself can alter the ability of neutrophils to produce NETs. Myocardial I/RI prompts the release of proinflammatory mediators that activate both the neutrophils and the coronary vascular endothelium. This activation promotes the
expression of adhesion molecules on both the neutrophils and endothelium, leading to adherence of neutrophils to the vascular endothelium, followed by trans-endothelial migration, neutrophil priming, and direct interaction with myocytes. Since the endothelial cells from human subjects with ALDH2 mutation adopt a proinflammatory phenotype, one can imagine that drugs inhibiting neutrophil adherence to and migration across monolayers of cytokine-activated endothelial cells could benefit these patients. Further studies should investigate the effects of ALDH2 and NETosis on other aspects of myocardial injury and repair, such as microvascular obstruction, inflammation, and fibrosis, as well as the interaction of ALDH2 with other genetic or environmental factors such as infections that affect NETosis and myocardial I/R.

Conducting clinical trials to test the efficacy and safety of NETosis/NETosis axis-targeting therapies in clinical trials should be explored. It is also essential to consider that ALDH2 seems to be a two-faced coin, and its role in STEMI is probably time dependent. While elevated ALDH2 activity is associated with reduced infarct size resulting in improved left ventricular function in animal models of acute myocardial infarction, overexpression of ALDH2 worsens ageing-induced cardiac hypertrophy and pressure overload-induced hypertrophy, possibly reflecting the dual functional repertoire of neutrophils in post-myocardial infarction inflammatory responses. Thus, there is a need to further establish the ALDH2/NETosis axis in a larger STEMI cohort with heart failure as an endpoint to relate individual NETosis levels found in patients to the risk of developing heart failure in the long term.

Overall, this study offers new insights into the cellular mechanisms of myocardial I/R and the potential therapeutic implications of modulating NETosis. It underscores the importance of considering the genetic background of the patients when developing personalized and precision medicine approaches for the treatment of cardiovascular diseases.

Declarations

Disclosure of Interest
The authors declare no disclosure of interest for this contribution.

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