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Translational Research

TRANSLATIONAL RESEARCH

Pharmacological activation of endogenous protective pathways against oxidative stress under conditions of sepsis

G. McCreath[1,†], M. M. F. Scullion[1,†], D. A. Lowes[1,2], N. R. Webster[1] and H. F. Galley[1,*]

[1]Academic Unit of Anaesthesia and Intensive Care, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, UK, and [2]Now at CXR Biosciences Ltd., Dundee DD1 5JJ, UK

*Corresponding author. E-mail: h.f.galley@abdn.ac.uk

Abstract

Background: Mitochondrial oxidative stress has a role in sepsis-induced organ dysfunction. The endogenous mechanisms to initiate protective pathways are controlled by peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1α) and nuclear factor erythroid 2-like 2 (NFE2L2). Activation of these pathways are potential therapeutic targets in sepsis. We used pharmacological activators to determine the effects on markers of mitochondrial damage and inflammation in human endothelial cells under conditions of sepsis.

Methods: Human endothelial cells were exposed to lipopolysaccharide plus peptidoglycan G to mimic a sepsis environment, with a range of concentrations of a selective synthetic agonist of silent information regulator-1 (SIRT-1) which activates PGC1α, or bis(2-hydroxy-benzylidene) acetone (2HBA) which activates NFE2L2, with and without inhibitors of these pathways. Cells were cultured for up to seven days and we measured mitochondrial membrane potential, metabolic activity, and density (as a marker of biogenesis), interkeukin-6 (to reflect inflammation) and glutathione (as a measure of antioxidant status).

Results: Under conditions mimicking sepsis, activation of the PGC1α and NFE2L2 pathways protected cells from LPS/PepG-induced loss of mitochondrial membrane potential (P=0.0002 and P=0.0009, respectively) and metabolic activity (P=0.05 and P<0.0001, respectively), and dampened interleukin-6 responses (P=0.003 and P=0.0001, respectively). Mitochondrial biogenesis (both P=0.0001) and glutathione (both P<0.0001) were also increased. These effects were blunted by the respective inhibitors.

Conclusions: The development of organ dysfunction during human sepsis is linked to mitochondrial dysfunction, and so activation of PGC1α/NFE2L2 is likely to be beneficial. These pathways are attractive therapeutic targets for sepsis.

Key words: antioxidants; endothelial cells; oxidative stress; sepsis

Sepsis is essentially a dysregulated and highly exaggerated systemic, inflammatory response to infection, accompanied by oxidative stress and mitochondrial dysfunction. In the developed world the incidence of sepsis continues to increase by around 10% annually, and now claims more lives than breast and lung cancers combined. Mitochondria are the major physiological producers of reactive oxygen species (ROS). During sepsis, mitochondrial ROS production exceeds antioxidant defences, leading to a state of oxidative stress that fuels inflammation and causes direct mitochondrial damage.[1] The resulting mitochondrial

† These authors contributed equally.

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Editor's Key Points

- Mitochondrial damage and dysfunction are characteristic features of sepsis-induced inflammation.
- Using a cellular model of sepsis, pharmacological activation of endogenous protective pathways preserved mitochondrial function and dampened inflammatory responses.
- Activation of endogenous cellular protective pathways is a promising therapeutic approach to sepsis, but further studies are necessary to translate *in vivo*.

dysfunction leads to further ROS release and initiates the same phenomenon, known as ROS-induced-ROS release, in neighbouring mitochondria. This self-perpetuating mechanism, resulting in widespread mitochondrial dysfunction and subsequent bioenergetic failure, is suggested to play a central role in sepsis-induced organ dysfunction,[2 3] and so therapeutic strategies to protect mitochondria during sepsis have been recognized as being important.[3–5]

Peroxisome proliferator-activated receptor gamma (PPARγ) co-activator 1-alpha (PGC1α) is a co-activator of a number of transcription factors responsible for controlling cellular metabolism.[6 7] Further transcription factors are under the control of PGC1α, such as nuclear factor erythroid-derived 2-like-2 (NFE2L2),[7] which regulates the expression of a number of protective mechanisms against oxidative stress, and GA binding protein transcription factor alpha (GABPA), which promotes activation of key transcription factors that control mitochondrial biogenesis. These events result in activation of protective cascades with generation of new mitochondria. A simple representation of the key pathways and the points of action of the agonists and inhibitors used in this study is provided in Figure 1.

PGC1α is regulated at both transcriptional and post-translational levels.[6 8] One of the various post-translational modifications that PGC-1α undergoes is deacetylation, catalysed by the enzyme silent information regulator-1 (SIRT-1).[8] As cellular energy levels decrease, SIRT-1 increases the activity of PGC-1α by removing acetyl groups. Under normal circumstances, SIRT-1 activity is regulated by the energy status of cells, but it can be also increased by synthetic agonists.[9]

NFE2L2 is present constitutively in the cell cytoplasm bound to a repressor protein called Kelch-like ECH-associated protein 1 (KEAP-1),[1] and its activation occurs when oxidant species react with cysteine in KEAP-1. This allows translocation of NFE2L2 into the nucleus where it binds to antioxidant response elements (ARE) to induce upregulation of key antioxidant enzymes. Agonists that act on the repressor protein KEAP can be used to activate NFE2L2.[10]

Activation of the PGC1α–NFE2L2 pathways are attractive potential therapeutic targets in sepsis. The aim of this study was to pharmacologically activate the PGC1α and NFE2L2 pathways using two different agonists and to determine the effects of these interventions on markers of mitochondrial damage and inflammation in human endothelial cells under conditions that mimic sepsis.

Methods

The agonists used in this study were 2-amino-N-cyclopentyl-1-(3-methoxypropyl)-1H-pyrrolo [2,3-quinoxaline]–3-carboxamide also known as SIRT-1-activator-3, a selective synthetic agonist of SIRT-1 that increases deacetylation of PGC1α,[9] and bis(2-hydroxy-benzylidene) acetone (2HBA) which is structurally related to curcumin[10] and acts as an agonist of NFE2L2, via effects on the KEAP-1 repressor protein. In addition, inhibitors were used

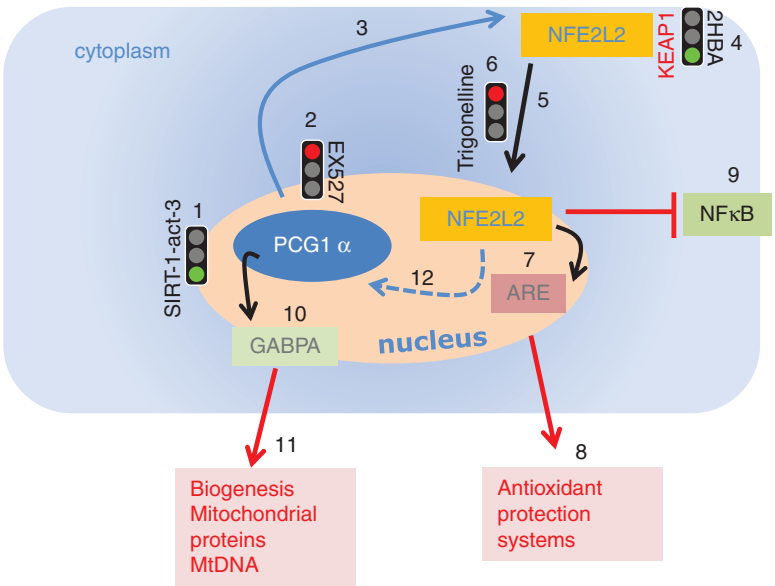

Fig 1 Very simplified depiction of the pathways described in this paper. PGC1α can be regulated post-translationally via SIRT-activator-3 (1) and this is inhibited by EX527 (2). Activation of PGCα co-activates NFE2L2 (3), which can also be activated by 2HBA via actions on its repressor protein KEAP-1 (4). When activated, NFE2L2 translocates into the nucleus (5) and this can be blocked by trigonelline (6). In the nucleus NFE2L2 activates ARE (7) which leads to upregulation of protective antioxidant pathways (8), and inhibits NFκB (9). PGCα also co-activates GABPA (10) leading to transcription of genes needed for biogenesis and synthesis of key mitochondrial proteins. NFE2L2 may in turn activate PGCα (12). For abbreviations see main text. Green traffic light indicates activator; red traffic light indicates inhibitor.