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Commentary

Therapeutic potential of tricarboxylic acid cycle metabolite itaconate in cardiovascular diseases

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Tricarboxylic acid cycle (TCA cycle) produces metabolites in the mitochondria under various pathophysiological conditions. Lipopolysaccharide (LPS), Toll-like receptor ligands, or interferons activate macrophages and disrupt TCA cycle. Macrophages then shift to a glycolytic phenotype and mitochondria function declines. These stimuli increase the expression of immune-responsive gene-1 (IRG1) that produces itaconate from cis-aconitate in mitochondria [1] (Fig. 1). Within the first 24 h after stimulation, succinate and itaconate accumulate and favor transcription factor hypoxia-inducible factor (HIF-1α) expression and stabilization. These metabolites and HIF-1α return to the baseline in 48 h [2].

IRG1 and itaconate are among the most highly induced enzyme and cell metabolite in activated human and mouse macrophages [3]. Itaconate shows similar structure to succinate and succinate dehydrogenase (SDH) inhibitor malonate. The first demonstration of itaconate anti-inflammatory activity came from a study showing that it counteracts the pro-inflammatory signals of succinate within the mitochondria by inhibiting the SDH activity, leading to succinate accumulation [3] (Fig. 1). In mitochondria, succinate enhances reactive oxygen species (ROS) production that acts as a pro-inflammatory redox signal to HIF-1α. After release to the extracellular space, succinate binds to its receptor SUCNR1 to activate the inflammatory pathway, with increased HIF-1α activity, enhanced pro-inflammatory cytokine (e.g. IL1β) expression, and reduced anti-inflammatory factor production [4] (Fig. 1). Itaconate also crosses the mitochondrial inner membrane to the cytosol where it uses its electrophilic α,β-unsaturated carboxylic acid to alkylate the cysteine residues ($Cys^{151,257,273,288,297}$) on Kelch-like ECH-associated protein-1 (KEAP1) that normally binds and promotes proteasome degradation of the nuclear factor erythroid 2-related factor 2 (Nrf2) [5], a transcription factor that was initially characterized as a sensor of oxidative stress. KEAP1 alkylation allows newly synthesized Nrf2 accumulate and migrate into the nucleus to activate the anti-oxidant and anti-inflammatory gene expression (Fig. 1). By binding to their promoters, Nrf2 inhibits the expression of pro-inflammatory genes IL1β and IL6 [6]. Itaconate also induces the expression of activating transcription factor-3 (ATF3) and ATF3-driven stress response in macrophages to repress inflammatory gene expression and mitochondrial stress (Fig. 1) [7]. In activated macrophages, IRG1 determines itaconate production. Activated bone-marrow-derived macrophages (BMDM) from *Irg1*$^{-/-}$ mice failed to produce itaconate [3]. Because of the SDH activity and insufficient itaconate, these cells showed abrogation of succinate accumulation, with increased cellular fumarate and malate concentrations [3] (Fig. 1). Nrf2 activation in response to LPS was also ablated in *Irg1*$^{-/-}$ macrophages [7]. While IRG1 mediates cis-aconitate to itaconate conversion, LPS increases IRG expression by inducing histone acetylation and methylation in the enhancer and promoter regions [8]. A fungal cell wall component β-glucan inhibits IRG1 expression by blocking this LPS activity, thereby restoring SDH expression in tolerant monocytes and reverting immunoparalysis [9]. Type-I interferon IFN-β enhances basal and LPS-induced IRG1 expression, which was blunted in BMDM from its receptor-deficient *Ifnar1*$^{-/-}$ mice [8]. In contrast, itaconate also inhibits the induction of type-I interferon in an itaconate-interferon regulatory loop (Fig. 1). Similarly, prevention of M2 macrophage polarization increases IRG1 expression and itaconate production. A decrease of IRG1 and itaconate by miR93 is required for M2 polarization. Therefore, both IRG1 and itaconaye are central regulators of cellular inflammation.

Endogenous itaconate is a relatively weak electrophile. The doubly charged itaconate is poorly taken up across the mitochondrial and plasma membranes unless there is a transporter such as dicarboxylate carrier, the one used by mitochondria inner membrane [8]. This can be overcome by cell permeable derivatives such as dimethyl itaconate (DI) and 4-octyl itaconate (OI) monoesters that are more hydrophobic than itaconate [8]. The anti-inflammatory function of itaconate and its derivatives correlate with their electrophilic

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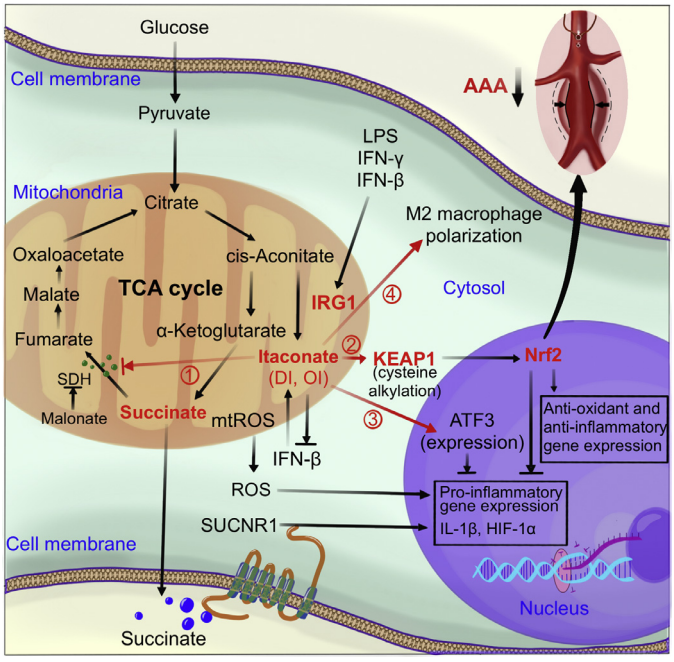

Fig. 1. LPS and interferons induce IRG1 expression and itaconate production from the TAC cycle. Itaconate and its derivatives DI and OI contribute to (1) succinate dehydrogenase inhibition and succinate accumulation; (2) KEAP1 cysteine alkylation, Nrf2 expression and nucleus translocation, and pro- and anti-inflammatory molecule expression regulation; (3) ATF3 expression; and (4) M2 macrophage polarization.

strength [9]. Therefore, both DI and OI have been used to enhance the itaconate activities in alkylating KEAP1, activating Nrf2, and driving Nrf2 and Nrf2-dependent gene expression [7,8]. DI triggers electrophilic stress that is usually controlled by glutathione. LPS-stimulated macrophages contain methylsuccinated glutathione that correlates with IRG1-dependent itaconate production [7]. The therapeutic potential of DI or OI has been tested in several disease models. In LPS-induced mouse sepsis, OI activated Nrf2, prolonged mouse survival, decreased clinical score, and decreased serum IL1β and TNF-α. These activities of OI were impaired in Nrf2-deficient or Nrf2-silenced macrophages [4,8]. In hepatic ischemia/reperfusion (IR) injury, IRG1 expression was increased in hepatocytes after I/R-induced oxidative stress. IRG1 deficiency enhanced liver injury. OI improved liver injury and reduced IR-induced hepatocyte death. Like in macrophages, Nrf2 was required for the IRG1 protective effect on hepatocytes against oxidative stress-induced injury. Itaconate slows TCA cycle metabolism and buffers redox imbalance by inhibiting the SDH activity and inducing anti-oxidative stress in astrocytes and neurons. In cerebral IR-injured mice, itaconate increased glutathione and reduced ROS with improved neuronal function. Mouse heart IR injury induced succinate oxidation. Inhibition of SDH with malonate reduced IR injury and mitochondrial ROS level and protected IR-induced heart attack. Intravenous injection of DI yielded over 40% reduction in infarct size than malonate did [3]. In cell cultures, DI reduced hypoxia-induced cardiomyocyte death and impaired LPS-induced BMDM mitochondrial ROS upregulation [3]. Therefore, the roles of itaconate and their derivatives are not limited to macrophages, but also hepatocytes, astrocytes, neurons, and cardiac cells.

In a recent issue of *EBioMedicine* [10], Song and colleagues reported elevated IRG1 and Nrf2 expression in human and murine abdominal aortic aneurysm (AAA) lesions. Although it was not tested whether AAA development increased lesion and plasma itaconate or succinate, daily peritoneal injection or minipump delivery of OI reduced angiotensin-II-induced AAA in mice. In contrast, shRNA-mediated IRG1 knockdown exacerbated AAA growth and lesion inflammation. As expected, OI promoted IFN-γ-induced macrophage Nrf2 expression and nucleus translocation and reduced macrophage inflammatory cytokine expression. IFN-γ lost these activities in IRG1 shRNA-treated macrophages. Instead, IFN-γ increased macrophage IL6 and IL1β expression. OI-induced Nrf2 expression involved KEAP1 Cys^{151} alkylation. Macrophages transfected with Cys151Ser mutant KEAP1 blunted OI-induced Nrf2 expression. Nrf2 suppressed IL6 and IL1β expression by direct binding to their promoters. Adeno-associated virus (AAV)-mediated overexpression of Nrf2 reduced AAA growth and lesion inflammation, but shRNA-mediated Nrf2 knockdown revealed the opposite phenotypes. Instead of using the *Irg1*$^{-/-}$ mice to test a role for itaconate in AAA, this study used AAV-mediated KEAP1 overexpression to increase AAA growth and lesion inflammation. OI treatment reversed these KEAP1 adverse activities, likely via KEAP1 alkylation and disassociation from Nrf2, although this study did not explore the details. This is the first study of TCA cycle metabolites in AAA that extends the concept of using OI or DI as a therapeutic regimen to slow murine and human AAA growth and associated lesion and systemic inflammation (Fig. 1). The demonstrated beneficial effects of OI and DI in sepsis, hepatic, cerebral, and cardiac IR injuries support this potential.

Declaration of Competing Interest

The author declares no conflicts of interest.

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References

[1] Degrandi D, Hoffmann R, Beuter-Gunia C, Pfeffer K. The proinflammatory cytokine-induced IRG1 protein associates with mitochondria. J Interferon Cytokine Res 2009;29(1):55–67.
[2] Seim GL, Britt EC, John SV, et al. Two-stage metabolic remodelling in macrophages in response to lipopolysaccharide and interferon-gamma stimulation. Nat Metab 2019;1(7):731–42.
[3] Lampropoulou V, Sergushichev A, Bambouskova M, et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metab 2016;24(1):158–66.
[4] Mills EL, Kelly B, Logan A, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 2016;167(2):457–70 e13.
[5] Ahmed SM, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta Mol Basis Dis 2017;1863(2):585–97.
[6] Kobayashi EH, Suzuki T, Funayama R, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun 2016;7:11624.
[7] Bambouskova M, Gorvel L, Lampropoulou V, et al. Electrophilic properties of itaconate and derivatives regulate the IkappaBzeta-ATF3 inflammatory axis. Nature 2018;556(7702):501–4.
[8] Mills EL, Ryan DG, Prag HA, et al. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature 2018;556(7699):113–7.
[9] Dominguez-Andres J, Novakovic B, Li Y, et al. The itaconate pathway is a central regulatory node linking innate immune tolerance and trained immunity. Cell Metab 2019;29(1):211–20 e5.
[10] Song H, Xu T, Feng X, et al. Itaconate prevents abdominal aortic aneurysm formation through inhibiting inflammation via activation of Nrf2. EBioMedicine 2020;57:102832. https://doi.org/10.1016/j.ebiom.2020.102832.