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-1\textalpha) expression and stabilization. These metabolites and HIF-1\textalpha 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]. Iaconate 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-1\textalpha. After release to the extracellular space, succinate binds to its receptor SUCLR1 to activate the inflammatory pathway, with increased HIF-1\textalpha activity, enhanced pro-inflammatory cytokine (e.g. IL1\beta) expression, and reduced anti-inflammatory factor production [4] (Fig. 1). Iaconate also crosses the mitochondrial inner membrane to the cytosol where it uses its electrophilic \(\alpha,\beta\)-unsaturated carboxylic acid to alkylate the cysteine residues (Cys151,257,273,288,297) 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 IL1\beta and IL6 [6]. Iaconate 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 \(\text{Irg1}^{-/-}\) 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 \(\text{Irg1}^{-/-}\) 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 \(\beta\)-glucan inhibits IRG1 expression by blocking this LPS activity, thereby restoring SDH expression in tolerant monocytes and reverting immunoparalysis [9]. Type-1 interferon IFN-\(\beta\) enhances basal and LPS-induced IRG1 expression, which was blunted in BMDM from its receptor-deficient Ifnar1\textsuperscript{-/-} mice [8]. In contrast, itaconate also inhibits the induction of type-1 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 itaconayte 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) monesters that are more hydrophobic than itaconate [8]. The anti-inflammatory function of itaconate and its derivatives correlate with their electrophilic
whether AAA development increased lesion and plasma itaconate or abdominal aortic aneurysm (AAA) lesions. Although it was not tested reported elevated IRG1 and Nrf2 expression in human and murine phages, but also hepatocytes, astrocytes, neurons, and cardiac cells. roles of itaconate and their derivatives are not limited to macro-

induced BMDM mitochondrial ROS upregulation[3]. Therefore, the reduction in infarct size than malonate did[3]. In cell cultures, induced heart attack. Intravenous injection of DI yielded over 40% reduced IR injury and mitochondrial ROS level and protected IR-
injury induced succinate oxidation. Inhibition of SDH with malonate and reduced ROS with improved neuronal function. Mouse heart IR SDH activity and inducing anti-oxidative stress in astrocytes and neu-

tates in AAA that extends the concept of using OI or DI as a 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 IR-

duced oxidative stress. IRG1 deficiency enhanced liver injury. OI improved liver injury and reduced IR-induced hepaticocyte death. Like in macrophages, Nrf2 was required for the IRG1 protective effect on hepatocytes against oxidative stress-induced injury. Iaconate slows TCA cycle metabolism and buffers redox imbalance by inhibiting the SDH activity and inducing anti-oxidative stress in astrocytes and neu-

rons. 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-

duced 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-

duced BMDM mitochondrial ROS upregulation[3]. Therefore, the roles of itaconate and their derivatives are not limited to macro-

phages, 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 Cys151 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 knock- 
down 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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