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Dimethyl Itaconate As A Novel Anti-Inflammatory Agent Targeting Tnf-A-Driven Nf-Kb Activation In Epithelial Cell Models

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ABSTRACT

Inflammation has a significant role in the pathogenesis of several epithelial diseases, leading to persistent inflammatory responses via the activation of the NF-κB signaling cascade, which TNF-α initiates. Dimethyl itaconate (DI), a cell-permeable derivative of itaconate, has recently been identified as an immunomodulatory agent that exhibits anti-inflammatory effects in human epithelial cells, as well as in other types of cells. In addition to delving into the creation of itaconate via IRG1, this article provides a comprehensive analysis of the function that endogenous and exogenous forms of itaconate, such as DI, play in the regulation of inflammation. We examine the metabolic pathways of itaconate and its derivatives, such as DI and 4-octyl itaconate (4-OI), to get a better understanding of the process of cellular absorption and reactivity of itaconate and its derivatives. In particular, we focus on the control of itaconate via IRG1. The major emphasis of our investigation is on the molecular pathways that are responsible for the impact of DI on the NF-κB signalling that is produced by TNF-α, particularly in epithelial cells. The process by which DI mediates transcriptional regulation involves the overexpression of ATF3, which in turn leads to a decrease in IκBν and a reduction in the generation of IL-6. In addition to this, DI is responsible for controlling important inflammatory genes and pathways, including JAK1-STAT6, TET2, and enzymes associated with glycolysis. Furthermore, it is responsible for stimulating the Nrf2 pathway by altering KEAP1. Moreover, it decreases the activity of inflammasomes. DI also helps reduce reactive oxygen species (ROS) in mitochondria and maintains antioxidant responses by inhibiting succinate dehydrogenase (SDH), a mechanism it employs to achieve this. Additionally, the research compares dimethyl itaconate to other itaconate derivatives and highlights the superior pharmacological properties of dimethyl itaconate. This is done in addition to addressing the therapeutic potential of dimethyl itaconate in the treatment of epithelial inflammation and related illnesses. Through the incorporation of information about metabolic regulation, transcriptional control, and immunological modulation, this work brings to light the potential of DI as a therapy for epithelial inflammation that is produced by TNF-α.

Keywords: Dimethyl itaconate (DI), TNF-α, NF-κB signaling, human epithelial cells, IκBζ, ATF3, KEAP1-Nrf2, inflammasome, IRG1, glycolysis inhibition, anti-inflammatory mechanisms, itaconate derivatives.

1 INTRODUCTION

1.1 Itaconate

After that, itaconate was named after the by-product of distilled citric acid that Samuel Baup discovered for the first time in 1836 by Crasso GL, as shown in Figure 1 (1). For the first time in 1932, Kinoshita discovered a filamentous fungus that produced itaconic acid (2). It was in 1945 that Kene's laboratory established the first industrial technique in the United States for the production of itaconic acid. (3). For a long time following its discovery, itaconate was predominantly used in the manufacturing of commercial polymers. This was because, in contrast to succinic acid and malic acid, scientists believed that itaconate did not have a significant influence on the metabolic processes of mammalian cells (4). However, over the course of time, researchers discovered that itaconate was able to effectively inhibit the activity of isocitrate lyase (ICL), which was responsible for reducing the development of bacteria under conditions when glucose was in short supply. Itaconate inhibited propionyl-coenzyme A (CoA) carboxylase (PCC), which in turn hindered the development of bacteria. This was accomplished without the presence of citrate lyase. Through the inhibition of the activity of SDH during infection, itaconate is effective in preventing the replication of the Zika virus (ZIKV) in the nervous system, as demonstrated by scientific experiments. (4). According to the findings of further study, itaconate was shown to possess powerful anti-inflammatory capabilities.

It was shown that macrophages generated a substantial quantity of itaconate when they were activated with either LPS or IFN-γ. Itaconate is generated by the catalytic decarboxylation of aconitic acid, which is encoded by immune-responsive gene 1 (IRG1) (5). This discovery was made by Michelucci and colleagues two years after the discovery of aconitic acid, which is an intermediate product of the TCA cycle. After the enzyme had been isolated, Dwiarti was able to identify specific characteristics of the enzyme before the year 2002. Researchers Lampropoulou et al. discovered in 2016 that animals missing the IRG1 gene generated more proinflammatory cytokines when stimulated (6). Furthermore, over the course of time, they revealed that itaconate had regulatory effects on anti-inflammatory cytokines. Later on, two major studies that were published in Nature revealed how itaconate modulates immunological responses and inflammatory cytokines. These studies expanded the breadth of the research and increased our understanding of the biological effects of itaconate (7). Our understanding of the function that itaconate plays in modulating macrophage metabolism via the Nrf2-independent ATF3 route and the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which is critical for regulating macrophage activity, has been expanded as a result of our study. Two pertinent research summaries were published in Nature Reviews Immunology in order to give feedback on the two studies that were previously conducted (8).

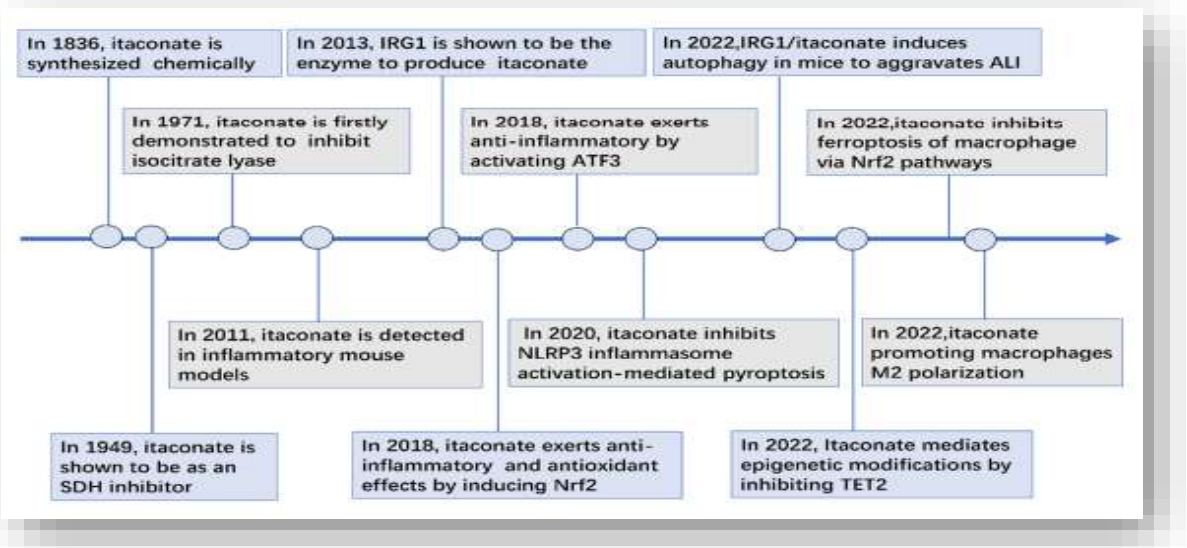


Figure 1: Itaconate discovery timeline [8]

Itaconate was chemically discovered as a result of citrate distillation in 1836. Itaconate inhibits SDH and isocitrate lyase, making it antibacterial in 1949 and 1971. In 2011, itaconate was found in several inflammatory models, and in 2013, IRG1 was proven to make it. In 2018, NRF2 activation showed antioxidant and anti-inflammatory properties (9). In 2018, it was proven that ATF3 activation reduces inflammation. Itaconate then activated autophagy, suppressed NLRP3 inflammasome activation-mediated pyroptosis, macrophage ferroptosis via NRF2 pathways, and TET2 inhibition-mediated epigenetic changes. In 2022, itaconate was shown to promote M2 macrophage polarization (10). Objective of this study: (i) To investigate dimethyl itaconate's (DI) anti-inflammatory effects on TNF-α-induced NF-κb signalling in human epithelial cells. (ii)To investigate the metabolic and molecular processes that DI affects that are related to inflammation of the epithelium.

2 METABOLIC PATHWAYS OF ITACONATE AND MODULATION OF IRG1

Providing metabolic energy and managing calcium homeostasis, mitochondria are the "cell powerhouses" of the cell. The TCA cycle converts acetyl-CoA to CO2 and collects electrons for the electron transport chain, which creates ATP in the mitochondrial matrix via enzymes. (11). The TCA cycle produces itaconate when not employed to generate energy. IRG1 and ACOD1 are additional names for cis-aconitate decarboxylase (CAD), which may convert it to itaconate as shown in **Figure 2**. Downregulation of isocitrate dehydrogenase in M1 macrophages causes citrate and isocitrate accumulation. Using 13C labelling, researchers discovered that M1 macrophages shifted carbon flow from α-KG production to itaconate creation.(12). Itaconate is produced by mitochondria; however, research on its translocation across membranes has shown inconsistent results. According to previous studies, activated macrophages exhibited millimolar itaconate levels, whereas M0 macrophages had hardly detectable amounts. According to Meiser et al. [10], RAW264.7 cells had an average extracellular concentration of 9 μM itaconate when activated with 10 ng/ml LPS, whereas BMDMs had an average concentration of 5 μM. Net release rates for RAW264.7 and BMDMs were 2.34 and 0.53 fmol/cell/h. Intracellular itaconate concentrations were 8 mM in RAW264.7 cells and 1.5 mM in BMDMs, explaining the release rate differences (13). Exogenous, unmodified itaconate also dramatically increased macrophage intracellular itaconate. Thus, passive diffusion may occur, but further study is needed. IRG1 in the mitochondrial matrix produces itaconate, which the 2-oxoglutarate carrier (OGC) transports across the mitochondrial inner membrane.

RAW264.7 cells with endogenous OGC deletion had lower cytosolic itaconate levels in response to LPS stimulation, indicating mitochondrial itaconate transport is essential for pool preservation. Itaconate may be broken down by succinyl-CoA synthetase to generate itaconyl-CoA, as shown in **Figure 3**. Hydrating itaconyl-CoA yields (S)-citramalyl-CoA, which is utilised to produce acetyl-CoA and pyruvic acid. Healthy people start with low IRG1 levels (14). Controlling IRG1 expression and producing itaconate is critical research. In response to LPS, LTA, BoNT/A, CpG-DNA, poly I: C, or KMRC011, IRG1 expression increases. Depletion of TLR2 or TLR4 in activated immune cells prevented TLR2, TLR4, and TLR9 from modulating IRG1 expression. TLR4 overexpression in macrophages boosted IRG1 expression. Myeloid Differentiation Primary Response Gene 88 (Myd88) encodes a cytosolic adaptor protein critical to the innate and adaptive immune response and a signal transducer in the interleukin-1 and Toll-like receptor signalling pathways (15). TLR4 signalling stimulates IRG1 gene expression via MyD88-dependent and MyD88-independent pathways, according to Hoshino K et al. TLR9, however, triggers IRG1 via MyD88. IRF3, IFNAR, and STATs signalling pathways promote IRG1 expression in MyD88−/− cells. During pathogen infections, IFN-1 and IFN-2 stimulate IRG1 expression, although IFNAR loss inhibits it. Shi et al. found that STAT1 deficiency inhibited Mtb-induced IRG1 expression. IRG1 is controlled by transcription and direct and indirect posttranscriptional mechanisms. Shi et al. found that miR-378 reduces IRG1 expression in human glioma cells. Protein kinase C (PKC) modulates protein activity by phosphorylating serine/threonine hydroxyl groups. PKC phosphorylates unknown substrates to regulate IRG1 expression. IRG1 mRNA expression was significantly reduced in RAW264.7 cells by PKC inhibitors H7 and genistein (Fig. 2). Apart from IRG1, pyruvate dehydrogenase (PDH)

transforms pyruvate to citric acid, making itaconate production necessary. LPS stimulation inhibits pyruvate dehydrogenase kinase 1 (PDK1) and enhances citric acid production, increasing itaconate synthesis (16). Catabolism converts itaconate to itaconyl-CoA and citramalyl-CoA in living organisms. The citrate lyase subunit beta-like (CLYBL) protein cleaves citramalyl-CoA into pyruvate and acetyl-CoA to restart the TCA cycle in LPS-activated RAW 264.7 cells (17).

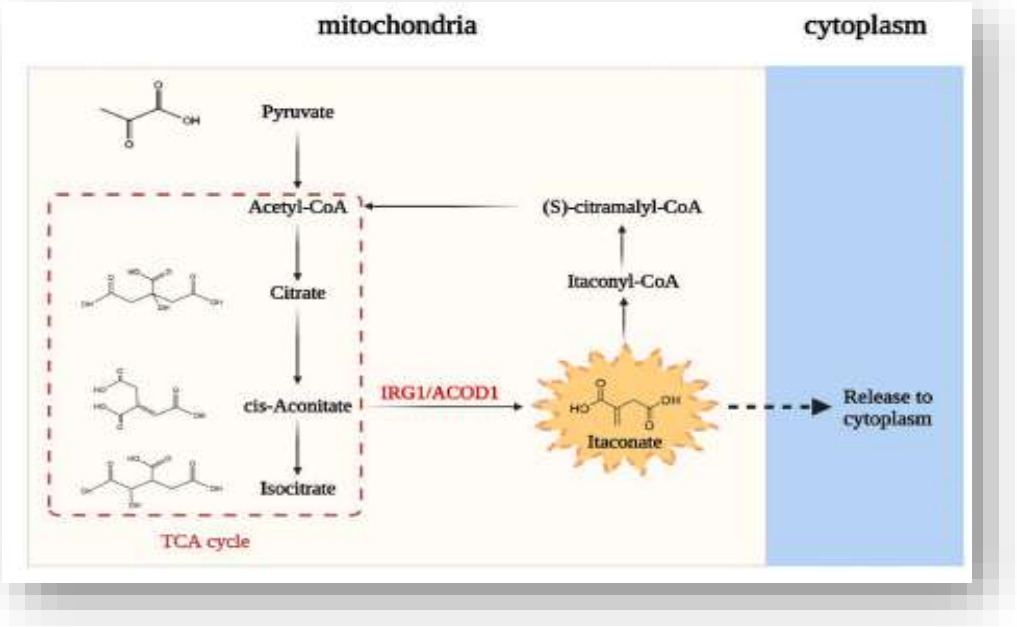


Figure 2: Investigation of the metabolism of itaconate. Bypassing the TCA cycle during energy generation produces the metabolite itaconate, which is formed in the mitochondrial matrix by the transformation of cis-aconitate to itaconate by the enzyme IRG1/ACOD1. Itaconate undergoes a series of conversions, the last of which catalyses the formation of acetyl-CoA, a compound that re-enters the TCA cycle. The TCA cycle includes the procedure shown in the brown rectangle.

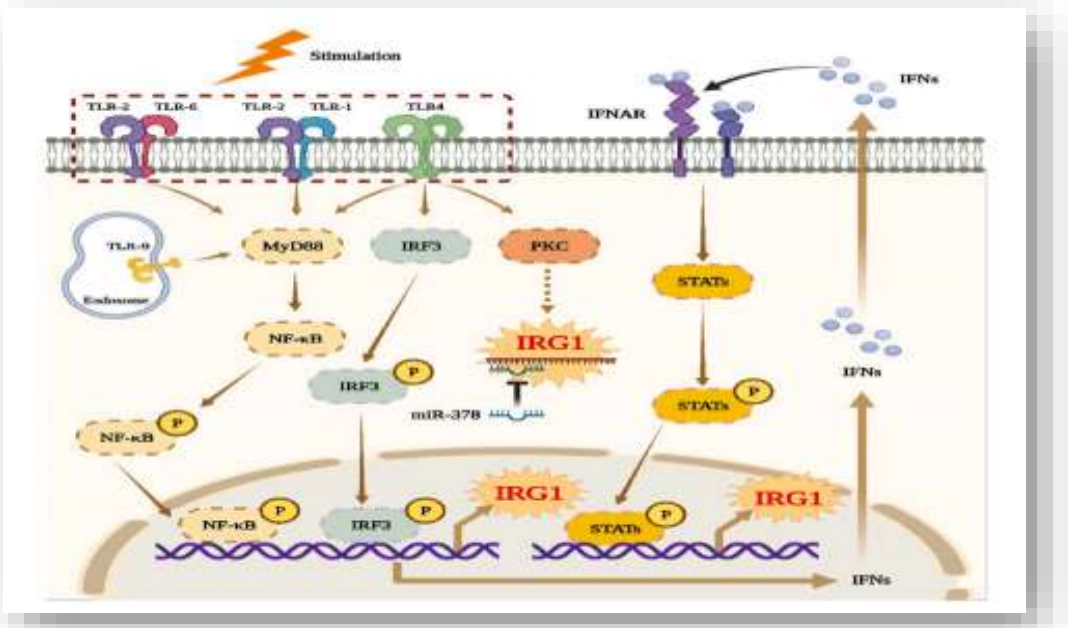


Figure 3 IRG1 modulation. Many stimuli activate receptors to express IRG1.
3 ITACONATE DERIVATIVE

After going through the Michael addition process on cysteine residues, itaconate suffers a change in the activity of the proteins that it binds to (18). There is a possibility that itaconate obtained from external sources will have difficulty traversing cell membranes and entering the cytoplasm due to the chemical composition of the substance, as shown in **Figure 4**. Dimethyl itaconate (4-I), 4-octyl itaconate (4-OI), and 4-ethyl itaconate (4-EI) are just a few examples of the itaconate derivatives that researchers have synthesised to demonstrate how the molecule functions (19). ItaCORMs, which are a mix of itaconate and carbon monoxide-releasing molecules, have also been explored for their potential anti-inflammatory effects (Fig. 3). Additionally, numerous biomaterials that are generated from itaconate have also been investigated for their potential anti-inflammatory attributes.

Researchers were able to produce dimethyl itaconate (DI) by first esterifying the carboxyl group that was present at the 1-position of itaconate. Due to the conjugation effect of the ester group, DI demonstrated enhanced reactivity in the Michael addition process, lowered negative charge, and impeded electron transport to a greater extent. (20). DI has been proven to have in vivo effects that are equivalent to those of dimethyl fumarate (DMF), according to research. These effects are achieved via activating Nrf2 and the genes that produce its downstream products. The expression of IκBν was decreased by DI, which in turn resulted in a reduction in the inflammatory response that was produced by LPS. (21). Nevertheless, some scientists say that DI is not an acceptable derivative because its electrophilicity and covalent modification, rather than its conversion to itaconate, are responsible for its extensive metabolic effects. For this reason, they believe that DI is not an appropriate derivative. Following this, Mills and colleagues came up with 4-octyl itaconate (4-OI), which was a derivative of itaconate that had an ester group at the distal end of an olefin. This was done to reduce the reactivity of mercaptan. In contrast to the impact that DI had, 4-OI was able to imitate the biological activity of itaconate by raising the amounts of itaconate in vivo via the process of hydrolysis. (22).

Although it is more polar and has less electrophilicity than diisocyanate (4-DI), 4-Ethyl itaconate (4-EI) has a structure that is equivalent to that of diisocyanate. Because, to the best of our knowledge, only one study has provided a comprehensive account of the biological effects of 4-EI, there is a pressing need for further investigation into the specific mechanism by which it inhibits inflammation (23). Because of the compound's capacity to release both itaconate and CO simultaneously, which was established via multidisciplinary research, the anti-inflammatory effects of ItaCORMs are superior to those of itaconate or CO alone in LPS-stimulated BMDMs. This is because ItaCORMs can release both itaconate and CO simultaneously. Additionally, itaconate has anti-inflammatory effects, and as a result, a number of researchers have started using it as a biomaterial (24). Scaffolds that are constructed of demineralised bone matrix and coated with itaconate are one example of an integrated scaffold that may assist in the repair of bone. In addition, they implanted PCL nanofibers that had been prepared with itaconate into animals to maintain the inflammatory response in injured areas. This was accomplished by virtue of the early proinflammatory effects of the nanofibers. Following a myocardial infarction (MI), nanofibers assist in the healing of damaged tissue and reduce the size of the infarct. In a similar breakthrough, researchers have developed injectable hydrogels that contain itaconate. The purpose of these hydrogels is to encourage the formation of cardiac stromal cells and to speed up the healing process after a myocardial infarction (25). As a result of the direct incorporation of itaconate into a polymer that preserves the biological activity of the chemical, recent research has shown that an itaconate polyester may be more effective than silica gel in reducing inflammation at both the duration and intensity levels. Every single one of these experiments contributes to our overall comprehension of the potential applications of itaconate in the treatment of illnesses in the years to come (26).

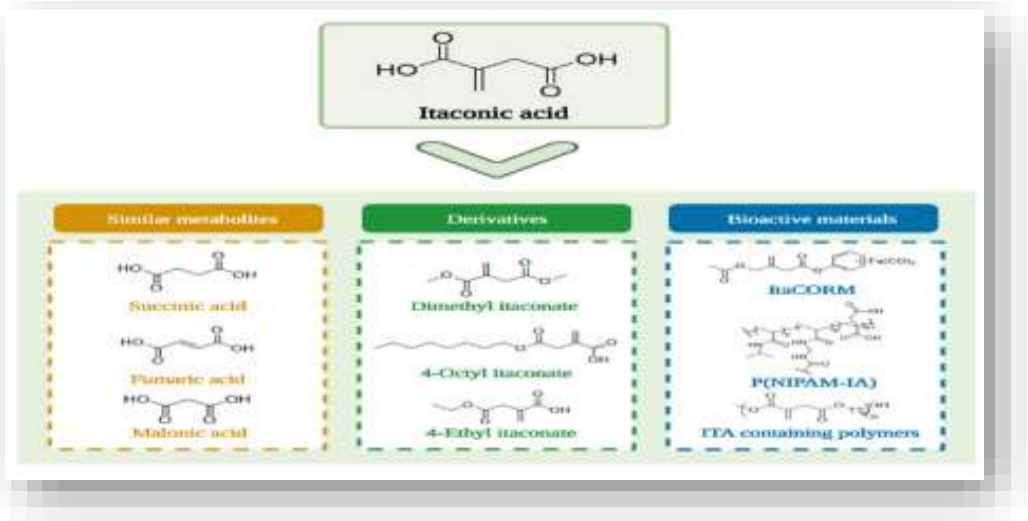


Figure 4 . Itaconate structure, metabolites, derivatives, and bioactive (14)

Succinate, fumarate, and malonate, which are structurally related to itaconate, have comparable biological activities. To exploit membrane permeability, researchers synthesised itaconate derivatives. Since multidisciplinary research has grown rapidly, ItaCORM, P(NIPAM-IA), and ITA-containing polymers have been synthesised to reduce inflammation. (27).

4 ITACONATE IN REGULATING THE INFLAMMATORY IMMUNE RESPONSE AND OXIDATIVE STRESS

The capacity of itaconate to modulate the immune system is one of the reasons why scientists are interested in working with it. IRG1−/− macrophages or IRG1−/− mice are the key instruments that scientists utilise to research the anti-inflammatory actions of itaconate in both laboratory and live animal settings (28). Itaconate and its derivatives are also used in this investigation. It is possible for the activities and functions of proteins to be influenced by the unsaturated double bond that itaconate has. This double bond enables it to covalently change cysteine residues via the Michael addition process (29). In particular, itaconate is principally responsible for controlling oxidative stress as well as inflammatory immunological responses via the mechanisms that are shown in **Figure 5**.

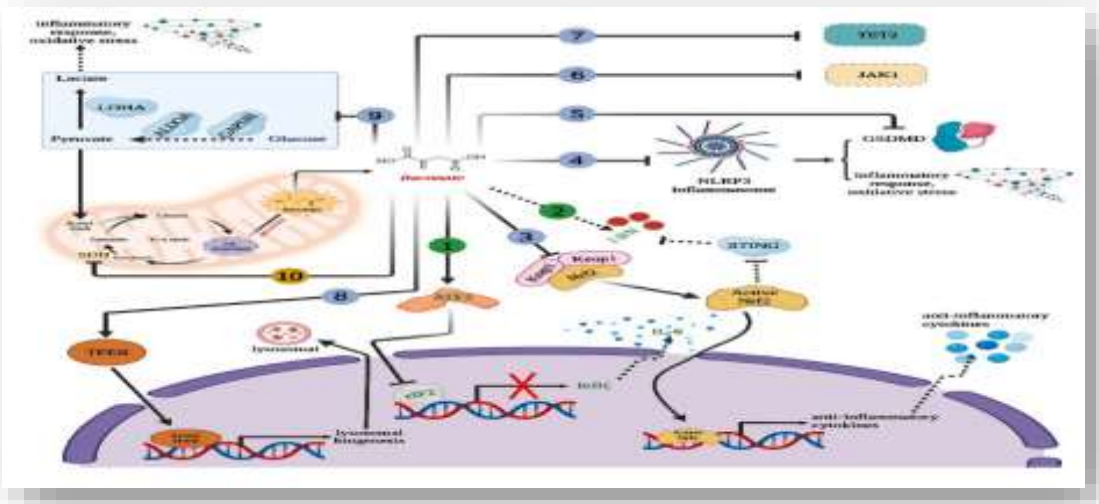


Figure 5: The traditional signalling mechanisms itaconate uses to control inflammation and oxidative damage [16].

Three kinds of classical signalling pathways govern inflammatory response and oxidative stress via itaconate: (1) ATF3/IκBζ axis regulation and type I IFN activation (① & ②); (2) KEAP1, inflammasome, JAK1-STAT6 pathway, TET2 catalytic activity, and TFEB nuclear translocation regulation

5 TRANSCRIPTIONAL REGULATION

5.1. Regulating ATF3/IκBζ axis activity

This gene is responsible for encoding the nuclear factor κB inhibitor zeta, also known as Nfκbiζ. In addition to being an immunomodulatory inhibitor and an essential regulator of IFN I activity, ATF3 is known to exert its regulatory influence on IFN I in a manner that is independent of the Nrf2 protein. (30). The knockdown of ATF3 has been demonstrated to result in an increase in the production of proinflammatory cytokines and IκBν in mouse embryonic fibroblasts, according to research. Furthermore, the suppression of IκBδ was achieved by itaconate and its derivatives. This was accomplished by elevating the expression of ATF3 and decreasing the production of the proinflammatory cytokine interleukin (IL-10) (31). According to the findings of research, BMDMs that were able to tolerate LPS showed secondary transcriptional responses that were downregulated by DI, although initial transcriptional responses were unaffected by DI immediately after LPS stimulation. About BMDMs that can tolerate IRG1−/− mice, it has been shown that the expression of IκBó is elevated after the second LPS treatment, as compared to BMDMs of the wild-type. (32). This research provides evidence that the control of IκBν takes place via the utilisation of endogenous itaconate. Furthermore, due to the fact that DI has electrophilic properties, researchers were able to produce a conjugate with glutathione. This chemical is capable of lowering reactive oxygen species (ROS). It is thus the case that itaconate and its derivatives can decrease the inflammatory response via the control of the ATF3/IκBν pathway. (33).

5.2. Regulation of type I IFNs

Macrophages that have been activated trigger the production of a large number of interferons (IFNs) in response to inflammation. To be more specific, several anti-infective characteristics are possessed by type I interferons. (34). It was found by Swain and colleagues that itaconate and its derivatives had different purposes when it comes to the regulation of type I interferon in the body. It was shown that the expression of type I interferon was effectively reduced by 4-OI and DI in macrophages that had been activated by LPS. (35). On the other hand, the level of type I interferons was significantly increased following pretreatment with unmodified itaconate. Taking into consideration these results, itaconate and its derivatives seem to serve unique roles in the regulation of type I interferons. (36). Following the injection of unmodified itaconate, this decreasing trend was effectively reversed, indicating that itaconate enhances the expression level of type I interferons in vivo. This is because the synthesis of type I interferons was lowered in IRG1−/− macrophages in an endogenous itaconate-deficient paradigm. There is a lack of knowledge about the specific method by which 4-OI blocked STING-dependent type I IFN production via Nrf2 activation. This question will need more exploration in further studies. (37).

6 PROTEIN MODIFICATION REGULATION

6.1. Regulating KEAP1 activity

In order for the E3 ubiquitin ligase enzyme KEAP1 to proceed with the degradation of Nrf2, it must first identify its substrate. In the face of oxidative stress and inflammation, Nrf2 transfers from the KEAP1-Nrf2 complex to the nucleus of cells. (38). Once there, it exerts its influence on the expression of genes that are dependent on the antioxidant response element (ARE) as well as genes that encode various detoxification enzymes. Several studies have shown that DI and 4-OI can activate Nrf2 and inhibit NF-κB, respectively, therefore contributing to the process of antioxidation. (39). Itaconate, which is a moderately electrophilic a, β-unsaturated dicarboxylic acid, has been used by researchers in order to alter the cysteine residue of KEAP1 using the Michael addition process. This change not only prevents the degradation of Nrf2 but also suppresses the action of KEAP1. (40). Additionally, the cysteine residues 151, 257, 273, 288, and 297 (C151, C257, C273, C288, and C297) in KEAP1 were modified as a result of the presence of 4-OI. Additionally, unmodified

itaconate, which displayed antioxidant and anti-inflammatory characteristics, was shown to affect KEAP1 in macrophages that had been activated by lipopolysaccharide (LPS) (41). This finding suggests that endogenous and exogenous itaconates control the KEAP1/Nrf2 pathway comparably. In addition, the activation of Nrf2 that was triggered by LPS was effectively inhibited by IRG1−/− macrophages. On the contrary, researchers observed that the presence of endogenous itaconate did not exacerbate the inflammation produced by particulate matter (PM) or the activation of Nrf2 in macrophages derived from IRG1−/− mice. (42). This was seen when PM was given to macrophages. Consequently, the experts who conducted this study believe that 4-OI is not as beneficial as endogenous itaconate. Itaconate and its derivatives have been demonstrated to considerably decrease inflammation and improve antioxidant responses, according to research that has been conducted. (43). To do this, they modify the cysteine residues of KEAP1, which in turn limits the activity of KEAP1 and activates Nrf2 in response to the stimulus supplied by LPS. (44).

6.2. Regulating inflammasome activity

Inflammation is characterised by inflammasome activity. By suppressing inflammasomes, inflammatory diseases may be addressed. Lampropoulou et al. found that LPS and ATP stimulation significantly decreased the expression of IL-1β and IL-18 in IRG1− /− BMDMs (45). Itaconate suppressed IL-1β expression without transcriptional interference, as shown by Swain et al. These findings suggest that itaconate directly controls NLRP3 to limit inflammasome activation. Hooftman et al.found that 4-OI produced changes to the cysteine 548 (C548) residue of NLRP3, resulting in NLRP3 binding to NEK7 and IL-1β production, independent of SDH and Nrf2. Gasdermin D is a downstream target of NLRP3 and a major pyroptosis molecule (46). In 2015, three groups studied GSDMD's pyroptosis regulation. GSDMD became a major factor in inflammatory responses and diseases after this discovery. Bambuskova et al. found that endogenoitaconate changed the cysteine 77 (C77) residue, blocking caspase-1 activation and GSDMD processing after long-term LPS priming, using proteome analysis. Late inflammasome activation may rely on GSDMD activity, despite its role in the NLRP3-caspase-1 axis (47). Research and drug development are focused on the NLRP3 inflammasome. Despite its arthritis treatment potential, MCC950, an NLRP3 inhibitor, failed in clinical trials. Inflammasome activity inhibitors like itaconate may treat inflammation-related illnesses since endogenous metabolites have fewer negative effects than synthetic drugs (48).

6.3. Regulating JAK1-STAT6 pathway activity

Due to the fact that M2 macrophages and T helper 2 (Th2) cells exhibit characteristics that are indicative of a type 2 inflammatory response, it has been hypothesised that M2 macrophages may play a pathogenic role in the development of asthma, pulmonary anaphylaxis, and pulmonary fibrosis (49). Both inflammatory diseases and the control of macrophage activation are affected by the JAK-STAT pathway, which is involved in the process. According to Runtsch et al., itaconate and 4-OI were able to inhibit the phosphorylation of JAK1 and STAT6, and they were also able to directly alter four cysteine residues on JAK1 (C715, C816, C943, and C1130). The last thing to mention is that itaconate and 4-OI were able to alleviate diseases that were caused by JAK1 modification-mediated macrophage M2 polarisation by suppressing this process (50).

6.4. Regulation of the catalytic activity of TET2

One of the most important metabolic intermediaries, α-KG, is essential for the TCA cycle. According to the findings of several studies, α-KG is a crucial component of TET2 that can establish hydrogen and ionic interactions with H1416, R1896, and S1898 inside the complex. (51). Furthermore, TET2 is responsible for controlling the formation of immune cells as well as their homeostasis and function. The findings that were given in this study led Chen et al. to the conclusion that itaconate, in a manner that is comparable to the impact of α-KG, inhibited the activity of TET2 by its direct binding to TET2. The fact that itaconate suppresses several proinflammatory genes in the NF-κB and STAT1 pathways may be the reason why it has an anti-inflammatory effect on macrophages that have been activated by lipopolysaccharide (LPS) (52).

6.5. Regulation of TFEB

An essential transcription factor, TFEB, is necessary for the process of lysosomal biogenesis. Following its connection with 14-3-3 regulatory proteins, TFEB stays in the cytoplasm after being phosphorylated at serine S211 by Mtor (53). This connection is necessary for TFEB to function properly. When the phosphorylation of TFEB is disrupted, it causes the protein to go into the nucleus, where it exerts its influence on the expression of genes that are linked with autophagy and lysosome generation (54). According to the findings of the research carried out by Zhang and colleagues, 4-OI was responsible for causing TFEB to change at Cys212, causing interference with the phosphorylation of TFEB, and causing TFEB to undergo nuclear translocation (55). In addition, nuclear TFEB was responsible for the elimination of germs and assisted in the process of lysosomal biogenesis during innate immune responses.

7 METABOLIC REGULATION

7.1. Targeting key glycolytic enzymes and inhibiting glycolysis

The states of energy metabolism in macrophages undergo significant changes in response to differences in immunophenotypes. An inflammatory response causes a decrease in the rate of oxidative phosphorylation and an increase in the rate of glycolytic metabolic activity in macrophages (56). In addition, when cells are subjected to oxidative stress, glycolysis generates a significant amount of lactate. Electrons are released as a result of an interaction between LDHA and NADH, which is the process that is being discussed here. (57). In the mitochondrial electron transport chain, these electrons are subsequently transformed into reactive oxygen species (ROS) (58). The topic of whether itaconate slows glycolysis and, as a result, increases the acquisition of anti-inflammatory and antioxidant phenotypes by macrophages has thus become a significant focus of inquiry (59). It has been shown via contemporary research that the regulation of aerobic glycolysis is the essential factor in producing the antioxidant and anti-inflammatory advantages that itaconate and its derivatives provide. A thiol-reactive probe was used by Qin et al. in order to measure chemoproteomic profiling of cysteine modifications that were generated by itaconate. Itaconate is responsible for the modification of 260 proteins, including an assortment of significant glycolytic enzymes such as ALDOA and LDHA. (59).

Through a modification of the cysteine residues of ALDOA and LDHA (C73 and C339) and C84, respectively, itaconate was able to reduce the catalytic activity of both of these compounds (60). Furthermore, the modifications in question increased the activity of ALDOA in IRG1−/− cells, while simultaneously reducing the rates of glucose consumption and lactate formation inside the cells (61). According to the findings of the research conducted by Liao and colleagues, 4-OI inhibited the enzymatic activity of GAPDH, alkylated its cysteine residue (C22), and reduced the rates of lactic acid production and extracellular acidification. After replacing the cysteine residue (C22) in GAPDH with an alanine residue, there was a significant reduction in the amount of IL-1β that was produced. Itaconate suppressed glycolysis and the inflammatory response by targeting GAPDH, which suggests that itaconate plays a crucial role in this process (62). This is comparable to the anti-inflammatory effects of heptelidic acid, which is a GAPDH-specific inhibitor.

7.2. Inhibition of SDH activity

SDH is a crucial metabolic enzyme in the triacylglycerol (TCA) cycle, which is responsible for the transformation of succinate into fumarate. In addition to being a major component of complex II of the mitochondrial respiratory chain, SDH is also an essential component of the TCA cycle. (63). When electrons are restored to complex I of the mitochondrial respiratory chain, reactive oxygen species (ROS) are produced (64). Additionally, succinate that has been stored is oxidised by succinate dehydrogenase (SDH), which leads to the formation of reduced coenzyme Q. Itaconate binds to the active site of SDH to prevent succinate from oxidising into fumarate. This is because itaconate and succinate share a position in the mitochondrial matrix and are structurally similar to one another. As a result, the generation of mitochondrial reactive oxygen species (mtROS) is inhibited, which is mediated by mitochondrial respiratory chain complex I (64). Additionally, the activity of SDH is blocked, which effectively controls the inflammatory response. Considering that

itaconate was able to decrease succinate levels while simultaneously causing a significant increase in oxygen consumption rates in LPS-stimulated IRG1− /− BMDMs, it seems to be an efficient SDH inhibitor (65).

8 ROLES OF ITACONATE IN INFLAMMATORY AND OXIDATIVE STRESS-INDUCED DISEASES

Numerous diseases associated with oxidative stress and inflammation are significantly affected by itaconate. In this article, we provide an overview of the roles that itaconate plays in several diseases, including inflammation brought on by autoimmune disorders, viruses, sepsis, damage caused by ischaemia-reperfusion, and illnesses brought on by oxidative stress (66).

8.1. Impact on autoimmune disease-induced inflammation

Autoimmune diseases usually affect the musculoskeletal system joints, muscles, bones, tendons, and ligaments, but may affect other organs. These conditions affect 5% of the world's population. Over 200 autoimmune illnesses have been identified, including Cryopyrin-associated Periodic Syndrome (CAPS), RA, psoriasis, MS, and SLE (67). Many autoimmune illnesses lower the quality of life over time. It's crucial to discover how to repair the immune system, which destroys tissue in autoimmune diseases. Traditional immunosuppression is the basis of autoimmune disease treatment, but it has several hazards, including infections and malignancies. Itaconate and its derivatives have shown potential in treating SLE, MS, psoriasis, RA, and CAPS. Using metabolomics, researchers have determined the metabolic spectrum of RA and SLE animals and humans. In SLE blood samples, Li et al. found a substantial reduction in itaconate expression. Michopoulos et al. examined blood, urine, and synovial fibroblasts at different stages of RA development and found that itaconate was significantly associated with disease progression and might be used as a disease marker. In Metabolites, two American and British teams examined plasma metabolite levels in RA patients. Both groups determined itaconate's role in RA (68). Itaconate and its derivatives are disease markers and potential autoimmune treatments. Tang et al. found that itaconate boosted the Nrf2 signalling pathway in THP-1 cells and SLE PBMCs, reducing inflammatory cytokines. 4-OI may also decrease IL-1γ release in isolated PBMCs from CAPS patients. Like parthenolide and oridonin, 4-OI may modify NLRP3 cysteine residues to protect them. In an animal model of experimental autoimmune encephalomyelitis (EAE), DI, a derivative of itaconate, suppresses MMP 3/9 synthesis, microglia activation, Th1/Th17 cell differentiation, and central axis nerve infiltration. Imiquimod promotes mouse psoriasis via TLR7/8 (69). DI has been used to treat these animals' pathogenic skin disorders. Imigquimod significantly increased IL-17A-mediated T cells in IRG1−/− mice. DMF is FDA-approved for numerous autoimmune illnesses. DMF treatment significantly improved SLE patients' clinical symptoms and quality of life. In this clinical research, DMF, alongside other drugs, had no adverse effects. Fumaric acid and itaconate, two DMF compounds, activated Nrf2 by changing reactive cysteine in KEAP1. DMF also hindered aerobic glycolysis by altering GAPDH. Itaconate and its derivatives may cure autoimmune illnesses due to their structural similarities to DMF (70).

8.2. Impact on virus-induced inflammation

Itaconate is crucial to virus-induced inflammation. THE COVID-19 epidemic has remained worldwide since the year's end. The SARS-CoV-2 virus has numerous methods to evade the immune system and reduce blood and lung immune cell counts. Autopsies of COVID-19 patients showed little viral infection but abundant immune cells. Two stages characterise COVID-19's pathogenic alterations. Rapid viral replication harms tissues quickly after infection (71). The second phase recruits numerous effector immune cells, causing local and systemic inflammatory reactions that last after the virus is gone. COVID-19 organ failure is usually induced by immune system overactivation. Many proteins in urine samples from 176 healthy controls, 55 pneumonia patients, and 86 COVID-19 patients were analyzed using proteomics. They found that COVID-19 patients, especially those with severe disease, have increased CLYBL, an enzyme that converts itaconate into acetyl-CoA. Metabolomics showed reduced itaconate plasma levels in severe COVID-19 patients, consistent with previous investigations. These data emphasise the necessity of itaconate in COVID-19 therapy. 4-OI directly inhibited SARS-CoV-2 replication. 4-OI controlled Nrf2 activity to minimise IFN overactivation, which lowered the virally-induced inflammatory response and improved COVID-19

symptoms. Itaconate was antiviral and anti-inflammatory against influenza A and ZIKV. In contrast, itaconate accelerates VSV replication. SIV infection in the body reduces the activity of m6A demethylase alkB homolog 5 (ALKBH5) and decreases the stability of α-ketoglutarate dehydrogenase (OGDH) mRNA and protein synthesis (72). Low OGDH levels reduced itaconate production, preventing virus replication in vivo. However, VSV infection with IRG1 increased inflammatory cell infiltration and lung damage in vivo. IRG1− /− animals showed effective suppression of VSV infection, and itaconate contributed to lung tissue damage via generating ROS and inflammatory cytokines during RSV infection (73).

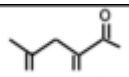
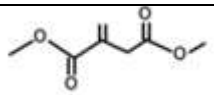
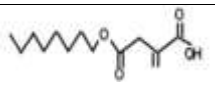
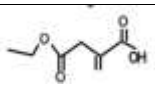
8.3. Impact on oxidative stress

Oxidative stress arises when ROS and RNS flow abundantly and damage cells (74). The body produces cytokines and DAMPs to fight oxidative damage. A recent study has shown that Nrf2 regulates oxidative stress and that itaconate largely controls the Nrf2 pathway. Heme oxygenase 1 (HO-1) and glutathione (GSH), downstream of Nrf2, protect against oxidative stress. Nrf2 also inhibits transcription by preventing oxidative stress cytokine promoter binding and RNA polymerase II recruitment. In 1 Regulating KEAP1 activity, itaconate dissociates and activates Nrf2 and alkylates KEAP1 cysteine residues 151, 257, 273, 288, and 297. Also increases KEAP1 degradation. Itaconate and its derivatives reduce oxidative stress in periodontal disease, liver fibrosis, vitiligo, pulmonary fibrosis, renal damage, osteoporosis, and neurological illnesses. Itaconate and DI activated Nrf2 with glutathione; itaconate activated Nrf2 to diminish oxidative stress in liver IRI in non-immune cells. (75).

9 ITACONATE DERIVATIVES

Itaconate, which is a five-carbon dicarboxylic acid (C5H6O4), has an α,β-unsaturated bond, which enables it to derivatise when it forms a bond with a nucleophile. Inactive proteins are produced as a result of the formation of a covalent connection between itaconate and cysteine residues on proteins during the Michael addition process. The chemical formula and structural features of the substance are shown in **Table 1**.

Table 1 Chemical structure and characteristics of itaconate and derivatives

Name	Molecular Structure	Chemical Formula	Molecular Weight (g/mol)	Permeability	Electrophilicity
Itaconate		$C_5H_6O_4$	130.10	±	±
DI		$C_7H_{10}O_4$	158.15	++	+++
4-OI		$C_{13}H_{22}O_4$	242.31	+++	++
4-EI		$C_7H_{10}O_4$	158.15	+	+

Due to their lower permeability across cell membranes, which is caused by their weak electrophilicity and relatively high polarity, dimethyl itaconate, 4-octylitaconate, and 4-ethyl itaconate are derivatives of the parent molecule itaconate. These derivatives may have more potential applications in the field of medicine or other medical applications. In addition to being referred to as the "powered-up" variation of itaconate, dimethyl itaconate has the chemical formula C7H10O4 as its chemical formula. Increasing the electrophilicity and membrane permeability of dimethyl itaconate is accomplished by the process of esterification at the carboxyl group located at position 1. Dimethyl itaconate may prevent lipopolysaccharide from reducing glutathione levels inside cells and from initiating the formation of IκBζ, which is an inhibitor of NF-κB transcription factors. As a

result of its rapid degradation and the fact that it does not undergo conversion to itaconate inside cells, its therapeutic usefulness is quite low. The chemical formula for 4-ethyl itaconate is C13H22O4, and it is a derivative of dimethyl itaconate that is less electrophilic and less easily transmembrane-transferable on the membrane. As a result of the esterification of just the 4-carboxyl group, the derivative is incapable of inhibiting IκBδ. The compound known as 4-octylitaconate is more comparable to itaconate[36]. Similar to dimethyl itaconate, it is capable of penetrating cell membranes and exhibits a little electrophilic nature owing to the length of its carbon chain. Esterases are unable to break it down, while other enzymes can hydrolyse it to itaconate regardless of whether or not lipopolysaccharide is present. 4-Ethyl itaconate is another itaconate derivative that has a high permeability to membranes. Its chemical formula is C7H10O4, and it is a compound that has a high permeability overall. The structure of 4-ethyl itaconate is similar to that of dimethyl itaconate; however, it is more polar and less electrophilic than dimethyl itaconate. Since it is just 4-carboxyl of dimethyl itaconate that is esterified, no effect on IκBδ can be considered an inhibitory effect. In the literature up to this point, 4-ethyl itaconate has been given a relatively restricted amount of attention. The use of itaconate and its derivatives is very effective in treating inflammatory and immune-related conditions. Individuals suffering from inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) might find relief from their symptoms via the use of itaconate, which has therapeutic properties (76). ALI is the core topic of this study, and the processes that it contributes to are the research's primary emphasis.

10 THERAPEUTIC MECHANISMS OF ITACONATE

10.1. Activating autophagy

The induction of autophagy has the potential to reduce the amount of tissue damage that is induced by ALI. Some proteins that are associated with autophagy are responsible for controlling the process of autophagy (77). In order to maintain the health of cells, it eliminates damaged cellular components. Microtubule-associated protein 1A/1B-light chain 3 (LC3) is a protein that plays a role in the process of autophagy. Through its collaboration with phosphatidylethanolamine, it is able to produce autophagosomes and take part in their maturation. This is accomplished by interacting with the membrane. In contrast, the inner and outer membranes of the autophagosome contain LC3-II, which is a lipidized form of LC3, whereas the cytoplasm has LC3-I, which is a soluble form of LC3. The progression from LC3-I to LC3-II, which is an essential stage in the process of autophagy, is a clear indication of autophagic activity. Adjusting the ratio of LC3-II to LC3-II may either promote or restrict autophagy activity, according to the consensus of these individuals. p62 is also recognized as a marker for autophagy breakdown, which is an additional achievement. The process of autophagy includes the engulfment and destruction of aggregates that are produced by misfolded proteins that are linked to p62 and tagged with ubiquitin. As a result, the buildup of p62 is indicative of a blockage in the route that leads to the breakdown of autophagy processes. The activation of autophagy has a number of effects, two of which are the reduction of inflammation in the lungs and the improvement of macrophage phagocytic activity. As an example, there is ABT-263, which is an inhibitor of the anti-apoptotic protein Bcl-2. This reduced sepsis in mice by enhancing the phagocytosis of bacteria by macrophages via a process that was reliant on beclin-1 autophagy. Additionally, autophagy may assist macrophages in transitioning from the M1 to the M2 phase and in reducing the release of acute phase inflammatory factors. It has been shown that itaconate leads to the activation of autophagy in macrophages. When itaconate was given to mice with acute lung injury (ALI), it was revealed that microglia that had been injected with LPS to imitate inflammatory processes went through the process of autophagy. This drug increased the levels of autophagy-promoting proteins LC3 and beclin-1 while simultaneously decreasing the levels of the autophagy inhibitor p62. The itaconate derivative 4-octylitaconate was shown to stimulate autophagy in an animal model of osteoarthritis. This was accomplished by a mechanism that included the downregulation of p62 (78). Through the inhibition of the PI3K/AKT/mTOR signalling pathway, this drug suppressed the expression of p62 in chondrocytes while simultaneously promoting the expression of LC3 and beclin-1. Although itaconate and its derivatives are known to trigger autophagy, the precise mechanism behind this phenomenon is not yet fully understood. Itaconate is able to prevent the phosphorylation of a specific serine, which is Ser211 in humans and Ser269 in

mice (79). Additionally, itaconate is able to activate transcription factor EB (TFEB) by directly alkylating a cysteine in the protein, which is Cys212 in humans and Cys270 in mice. Because of this, the kinase mTORC1 is unable to phosphorylate the serine, which would result in the inactivation of TFEB. As a consequence of this, lysosomal autophagy is improved because the constitutively active transcription factor drives the expression of a large number of genes that increase the creation and activity of lysosomes.

10.2. Inhibiting pyroptosis

Pyroptosis, another non-apoptotic cell death, promotes ALI. NLRP3 inflammasome inflammation causes pyroptosis. Studies demonstrate that itaconate and its derivative 4-octylitaconate may inhibit the NLRP3 inflammasome in macrophages, reducing inflammatory cytokines (80). A cysteine in NLRP3 (Cys548 in HEK293T cells) is alkylated in 4-octylitaconate, preventing binding to NIMA-related kinase 7 and reducing IL-1β production. Pyroptosis prevention seems to include this pathway. The compound 4-octylitaconate prevents pyroptosis by reducing the activity of signalling pathways such as NF-κB and MAP kinases in RAW264.7 and mouse bone marrow-derived macrophages, and STING and IRF3 in a mouse model of acute respiratory distress syndrome. 4-octylitaconate alkylates cysteine147 in STING in HEK293T cells. This alteration reduced STING phosphorylation and proinflammatory molecule production, including IFN-β, TNF-α, IL-1b, and IL-6. A derivative was found to alkylate STING cysteine 91 and impede palmitoylation. Evidence suggests that it may inhibit pyroptosis caused by GSDMD and GSDME in mice with acute colitis, as shown in previous research. GSDMD and GSDME drive pyrotosis. Itaconate regulates macrophage protein GSDMD at cysteine77, inhibiting caspase-1 activation and processing and preventing pyroptosis. Itaconate and its derivatives affect the NLRP3 inflammasome via many routes. For instance, 4-octylitaconate induces NRF2 to activate antioxidant response element-regulated genes. Heme oxygenase, glutathione peroxidase 4, and NAD(P)H dehydrogenase genes neutralise mitochondrial ROS. NRF2 stay inactive in the cytoplasm via binding to Kelch-like ECH-associated protein 1. However, 4-octylitaconate alkylation at human protein cysteines 257, 288, and 273 activates and translocates NRF2 into the nucleus. NRF2 is normally maintained low by interacting with KEAP1, leading to its degradation and release. After then, free NRF2 nuclear translocation activates genes that produce anti-inflammatory and anti-oxidative proteins. Itaconate modulated mitochondrial metabolism and architecture to lower airway inflammation. It reduced mitochondrial ROS release, stopped NLRP3 inflammasome activation, and controlled mitochondrial fusion/fission in a mouse model of allergic airway inflammation (81).

10.3. Inhibiting ferroptosis

A significant reduction in lung damage was seen in the human bronchial epithelial cell line BEAS2B when ferrostatin-1 was used to inhibit ferroptosis(82). This finding suggests that there may be a possible connection between ferroptosis and the development of acute lung injury (ALI). Cell death is a process known as ferroptosis, which takes place when reactive oxygen species (ROS) accumulate and iron-dependent lipid peroxidation takes place (83). These processes in the bronchial epithelium were made worse when they were exposed to a mouse model of acute lipopolysaccharide-induced inflammation (ALI). This is because lipopolysaccharide raises the levels of iron in lung tissues, lowers glutathione levels, and inhibits the activity of glutathione peroxidase 4, which normally neutralises reactive oxygen species (ROS) and repairs damage to membranes (84). Itaconate alleviates acute lung injury (ALI) by a mechanism that involves the suppression of ferroptosis. The activation of the NRF2 pathway, the regulation of glutathione levels, the suppression of reactive oxygen species formation, the reduction of lipid peroxidation, and the reduction of inflammatory factor release are all assumed to be involved in this process. A good illustration of this is the activation of glutathione peroxidase 4, the transcription factor NRF2, and the cysteine/glutamate transporter SLC7A11 in human THP-1 monocytes that were treated with 4-octylitaconate and then lipopolysaccharide (85). It seems that there is an enhanced capacity to lessen the damage caused by ferroptosis, as well as a reduction in the levels of reactive oxygen species (ROS) and malondialdehyde, which are essential indicators of oxidative stress. In a study that provided evidence for the role of NRF2 in this process, it was discovered that the expression of glutathione peroxidase 4 (GPX4) was reduced when Nrf2 was silenced (86).

Additionally, research has shown that GM-CSF can activate the transcription factor C/EBPb, which in turn leads to an increase in the expression of ACOD1 in neutrophils (87). Additionally, this results in an increase in the expression of NRF2-dependent antioxidant response genes such as Gpx4, Gclc, and Nqo1, which in turn mediates resistance to ferroptosis. When everything is taken into consideration, our findings indicate that the NRF2 pathway is the mechanism by which itaconate inhibits ferroptosis from occurring (88). Because of its structural similarities to succinate, itaconate has the potential to reduce the production of reactive oxygen species (ROS) in mitochondria by inhibiting succinate dehydrogenase (SDH) in a competitive manner. In a mouse model of acute respiratory distress syndrome (ARDS), the derivative 4-octylitaconate enhanced macrophage pyroptosis and eased symptoms by lowering such production and preventing the oxidative stress-induced release of mitochondrial DNA into the cytoplasm of alveolar macrophages (89). This was accomplished by reducing the production of pyroptosis and facilitating the release of mitochondrial DNA. It seems that the ability of 4-octylitaconate to reduce the levels of reactive oxygen species (ROS) in macrophages is associated with the regulation of the signalling p38MAPK (90). By promoting autophagy, itaconate has the potential to aid in the prevention of ferroptosis. On the other hand, ferroptosis may be brought about by ironophagy, which is a subtype of autophagy called ironophagy (91). Ironophagy can be brought about by high levels of itaconate. It is necessary to research the possible impacts that itaconate may have on ferroptosis and ALI, as well as the conditions that may cause this inhibition or induction to take place (92).

10.4. Promoting polarization of macrophages to an anti-inflammatory M2 phenotype

Itaconate and its derivatives have the potential to change the polarisation of macrophages, causing them to transition from an M1 to an M2 phenotype. Four-octylitaconate was shown to lower the expression of the M1 macrophage marker CD68 in a mouse model of osteoarthritis, while simultaneously increasing the expression of the M2 markers Arg-1 and CD206 from the same mice model (93). In combination with these modifications, it was discovered that cartilage deterioration and synovial inflammation were less severe than they often were. Through the administration of 4-octylitaconate to macrophage cells, the M1 indicators CD86 and inducible nitric oxide synthase were found to be downregulated, whilst the M2 markers CD206 and Arg-1 were found to be increased. On the other hand, Runtsch and colleagues discovered that the 4-octylitaconate derivative would reduce the phosphorylation of the JAK1 enzyme in M2 phenotype macrophages, which would result in the inhibition of the enzyme's expression. In the method, the 4-octylitaconate derivative has the potential to directly modify the cysteine residues that are located on JAK1 at the main locations of 715, 816, 943, and 1130. In research that Blanco and colleagues carried out, it was shown that the derivative 4-octylitaconate has the potential to reduce some of the symptoms that are associated with rat lupus. It is possible that this influence might be explained by the increase in regulatory T cells (Tregs) and CD8+ T cells, as well as the decrease in type I interferon and inflammatory cytokines. There is a possibility that itaconate considerably improves acute lung injury (ALI) via controlling the immune response by having an effect on both M1 and M2 macrophages. There is a need for more research into the mechanisms by which itaconate influences the polarisation of macrophages.

10.5. Inhibiting neutrophil activation

There is a significant role that neutrophils play in the development of acute lung injury. Investigations are now being conducted on the interactions that neutrophils have with itaconate and its derivatives. For instance, mature neutrophils that circulate in the bloodstream have the potential to produce itaconate and exhibit robust inflammatory activity in response to trauma (94). Furthermore, they have the capacity to transport itaconate that is already present in the body to the bone marrow, where it has the potential to trigger the production of immune cells known as granulocytes and monocytes. Exogenous itaconate has the potential to reduce inflammation and heterotopic ossification at injury sites, hence enhancing tendon differentiation and healing. A mouse model of lung infection caused by Staphylococcus aureus showed that itaconate slowed down the glycolysis of neutrophils and increased the number of neutrophils that died (95). In addition to this, it inhibited the oxidative phosphorylation of neutrophils and specifically targeted nicotinamide adenine dinucleotide phosphatase, namely NADPH oxidase. As a result of the derivative 4-octylitaconate's ability to enhance the expression of NRF2/Heme Oxygenase (HO-1) and reduce the expression of

hypoxia-inducible factor 1α (HIF-1α), it was able to inhibit the growth of nuclear endothelial cells (NETs) in a model consisting of mice that were either normal or obese (96).

10.6. Regulating epigenetic modifications

DNA methylation and histone modification are epigenetic alterations that influence gene expression without changing the DNA sequence. Epigenetic changes are important in ALI/ARDS pathogenesis. Epigenetic regulator enzyme "ten-eleven translocation methylcytosine dioxygenase 2" (TET2) cannot convert DNA 5-methylcytosine to 5-hydroxymethylcytosine with the byproduct 4-octylitaconate. Blocking this modification reduces inflammation by downregulating genes upregulated by cytokines through transcription factors NF-κB and STAT1, resulting in less inflammatory cytokines (IL-6, Cxcl9, Cxcl10, and Cxcl11). Aso et al. found that itaconate regulates T cell subsets epigenetically. It promotes Treg cell development and inhibits Th17 cell differentiation. It demethylates histones via inhibiting methionine adenosine transferase and isocitrate dehydrogenase 1 and 2 (IDH1/2), affecting the chromatin accessibility of key transcription factors at Il17a and Foxp3 locations. Domínguez-André also found that itaconate affects histone 3 lysine 27 acetylation. In ALI, dysregulated histone deacetylation changes gene expression, compromising lung epithelial integrity and worsening damage. Lipopolysaccharide-induced lung endothelial damage may be mitigated by histone deacetylase inhibition. Valproic acid inhibits histone deacetylases, upregulating Irg1 and increasing itaconate synthesis, which protects tissue against inflammation and oxidative damage. Future research should examine if itaconate supplements preserve pulmonary endothelium in ALI.

10.7. Inhibiting glycolysis

With the appropriate reduction of glycolysis, the progression of acute lung injury (ALI) may be slowed down. As an illustration of this principle, the glycolysis inhibitor 2-deoxyglucose significantly decreased lung tissue damage and neutrophil production in an animal model of acute lung injury (ALI), while concurrently downregulating pro-inflammatory markers (97). Itaconate and its derivatives have the potential to block a wide variety of enzymes, which may result in the suppression of aerobic glycolysis. These enzymes include glyceraldehyde-3-phosphate dehydrogenase (GAPDH), aldolase A, and lactate dehydrogenase, which is the enzyme that is responsible for rate-limiting enzyme in the whole glycolytic process (98). The inhibition of each enzyme is caused by the alkylation of cysteines 245 in mouse GAPDH, 73 and 339 in mouse aldolase A, and 84 in mouse lactate dehydrogenase.

11 MOLECULAR PATHWAY-RELATED GENES AND THEIR INTERRELATIONSHIP

There are a number of genes and molecular pathways that have shown the crucial roles that itaconate plays in the treatment of acute lung injury. Itaconate is responsible for activating a number of essential genes, one of which is nuclear factor erythroid 2-related factor 2 (Nrf2) (98). The activation of Nrf2, which in turn leads to an increase in the expression of antioxidant genes, has the potential to bring about a reduction in the amount of oxidative stress and inflammation that occurs in the lungs. According to research, itaconate has the potential to impact inflammatory responses via altering the STAT3 gene, which stands for signal transducer and activator of transcription 3 (100). Not only does itaconate have the capacity to impact other molecular pathways, but it also can regulate the NLRP3 (NOD-like receptor family pyrin domain-containing 3) inflammasome pathway. Itaconate is able to decrease inflammation in the lungs by inhibiting the activation of NLRP3, which in turn inhibits the production of cytokines that are responsible for promoting inflammation. Through its interaction with the Keap1-Nrf2 pathway, it may also aid in the enhancement of antioxidant defence mechanisms. Itaconate may also have an effect on the mitochondria's metabolism and function, which is important since mitochondria are necessary for maintaining cellular homeostasis and responding to lung injury (101). The interaction between these genes and molecular pathways, which are responsible for the therapeutic effects of itaconate in acute lung injury, provides a more in-depth understanding of the mechanisms that are at play and the possible therapeutic intervention targets that may be used (102).

12 CONCLUSION

As a result of the discovery of itaconate and its derivatives, we have gained a significant amount of knowledge on metabolic immunoregulation, particularly in relation to epithelial inflammation. Among the derivatives, dimethyl itaconate (DI) has garnered significant interest as a potent anti-inflammatory medication. This is mostly due to its ability to suppress the NF-κB signalling pathway in human epithelial cells, which is triggered by TNF-α. Through its unique ability to modulate multiple cellular pathways—including transcriptional regulation (ATF3/IκBζ axis), protein modification (KEAP1–Nrf2 activation and inflammasome suppression), and metabolic reprogramming (glycolysis and SDH inhibition)—DI offers a multifaceted approach to dampen pro-inflammatory responses. In conditions where chronic inflammatory stimuli, including tumour necrosis factor-alpha (TNF-α), disrupt the integrity of the epithelial barrier and perturb the equilibrium of the immune system, these pathways assume a greater significance. In addition, as compared to other itaconate derivatives, DI has a significant therapeutic potential due to the fact that it has better regulatory efficiency, stability, and cell permeability and is thus more effective. Considering the modulatory effects that it has on inflammation, oxidative stress, and immune cell polarisation, more research must be conducted to investigate its potential use in the treatment of inflammatory and autoimmune epithelial diseases. The chemical compound known as dimethyl itaconate has significant importance in the field of immunometabolism. This is because it presents novel opportunities for the development of tailored anti-inflammatory therapies for epithelial disorders that are caused by the activation of TNF-α and NF-κB.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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