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REVIEW

The Role and Mechanism of Metformin in Inflammatory Diseases

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Abstract: Metformin is a classical drug used to treat type 2 diabetes. With the development of research on metformin, it has been found that metformin also has several advantages aside from its hypoglycemic effect, such as anti-inflammatory, anti-aging, anti-cancer, improving intestinal flora, and other effects. The prevention of inflammation is critical because chronic inflammation is associated with numerous diseases of considerable public health. Therefore, there has been growing interest in the role of metformin in treating various inflammatory conditions. However, the precise anti-inflammatory mechanisms of metformin were inconsistent in the reported studies. Thus, this review aims to summarize various currently known possible mechanisms of metformin involved in inflammatory diseases and provide references for the clinical application of metformin.

Keywords: metformin, AMPK, inflammation, mechanisms

Introduction

In the 19th century, metformin (1,1-dimethylbiguanide hydrochloride) was discovered, which is a biguanide derivative extracted from the plant Galega officinalis (French lilac).[1] By promoting the binding of insulin and receptors, metformin accelerates glucose utilization in the body, reduces the output of liver sugar, improves insulin resistance, and achieves the therapeutic effect of controlling blood sugar.[2] With its economy, safety, and effectiveness advantages, it has become the first-line drug for type II diabetes. In recent years, several experimental studies and epidemiological data have shown that, in addition to hypoglycemic effects, long-term use of metformin may exert anti-inflammation,[3] avoid age-related diseases,[4] prevent tumors,[5] and reduce the occurrence and development of various metabolic diseases.[6] Notably, given its potential anti-inflammatory properties, metformin is now receiving increasing attention.

Inflammation is a defense mechanism against various noxious stimuli triggered by exogenous stimuli, such as infections[7] or endogenous sterile injuries.[8] Overactive inflammatory responses, however, can be harmful to the body. Studies have found that inflammation is related to the development of numerous diseases. Chronic inflammation can induce senescence,[9] tissue destruction,[10] immunosuppression,[11] and cancer.[12]

Although metformin is widely used in the clinic, especially for controlling inflammatory diseases, and has made promising progress, the mechanisms behind its anti-inflammatory effects are still poorly understood. Hence, in this review, to clear the way for possible targets for therapeutic use, we will concentrate on current developments in research on the function of metformin in various inflammatory illnesses and its underlying mechanisms of action.

Adenosine Monophosphate-Activated Protein Kinase (AMPK)-Dependent Pathways

Metformin is a medication often prescribed for individuals with diabetes. It works to reduce the production of glucose in the liver through both AMPK-dependent and AMPK-independent pathways.[13] Similarly, metformin also inhibits inflammation in an AMPK-dependent[14] and AMPK-independent manner.

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AMPK is a heterotrimeric serine/threonine protein kinase consisting of three subunits. It serves as a crucial metabolic sensor linking cellular energy homeostasis to the regulation of inflammatory signaling.[15–17] Indeed, AMPK is activated when energy is deficient, with an increase in the adenosine monophosphate (AMP)/ adenosine triphosphate (ATP) ratio being the activation signal.[18] To ensure metformin's effectiveness, activating the AMPK system is vital. By inhibiting complex I of the mitochondrial respiratory chain, also known as the electron transport chain (ETC), metformin inhibits transmembrane electron flow and membrane potential formation, thereby reducing mitochondrial oxygen consumption and inhibiting the production of ATP, increasing the AMP/ATP ratio and thereby activating AMPK.[19,20]

Through activation of AMPK, energy homeostasis is restored by promoting catabolic pathways, which cause ATP synthesis, and inhibiting anabolic ways that consume it. In addition, metformin suppressed inflammatory cytokine production by promoting macrophage polarization to the anti-inflammatory M2 type via activating AMPK-1α phosphorylation.[21] Besides, its anti-inflammatory properties appear related to its inhibitory effect on nuclear factor-kappaB (NF-κB), the most common transcription factor for inflammatory responses. Moreover, activating AMPK facilitates the nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and increases the expression of heme oxygenase-1 (HO-1) to counteract lipopolysaccharide (LPS)-induced cellular damage.[22,23]

To date, various models have been used to assess the metformin effects on renal inflammation. AMPK activation appears to be a key mechanism behind many of these renoprotective effects.[24–29] Moreover, in an experimental study of myocardial ischemia-reperfusion (I/R) injury, the metformin treatment increased the phosphorylation of AMPK, decreased pro-inflammatory cytokines, and suppressed NLRP3 inflammasome activation. Then all led to a significant reduction in myocardial infarction size, apoptosis attenuation, and myocardial fibrosis inhibition. Significantly, the observed effects were reversed upon inhibition of AMPK phosphorylation by Compound C (CC).[30] As shown in a mouse model, metformin significantly reduced lung inflammation in SiO_2-instilled mice at the fibrotic stages, which can be exacerbated by inflammatory mediators, including interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α).[31] Besides, metformin attenuated oxidative stress-induced cell toxicity, the epithelial-mesenchymal transition process in epithelial cells, and inhibited inflammation response in macrophages via an AMPK-dependent pathway.[32] Another AMPK-dependent effect of metformin shown in research was interrupting the priming signal for NLRP3 inflammasome activation via blockade of Toll-like receptor 4 (TLR4)/NF-κB signaling pathway, resulting in the inhibition of bioactive forms IL-1β and IL-18 as well as the pyroptosis process.[33]

Additionally, it was discovered that metformin could induce autophagy through AMPK activation, resulting in an increase of Beclin-1 and a decrease of p62/SQSTM1, ultimately leading to the degradation of a critical inflammatory mediator, NLRP3.[33] This is similar to the findings of Fei et al.[34] In another study, metformin could decrease STAT3 phosphorylation through an increase in AMPK activity, which in turn inhibited differentiation of monocyte to macrophage and eventually suppressed proinflammatory responses. Moreover, by this mechanism, metformin attenuated aortic atherosclerotic lesions induced by AngII and aneurysm in ApoE−/− mice.[35]

Collectively, evidence suggested that metformin exerts remarkable anti-inflammatory and immunosuppressive effects in inflammatory/autoimmune models. A vital component of this process was enhancing the AMPK pathway.

Liver Kinase B1 (LKB1)

LKB1 is a serine/threonine kinase encoded by the tumor suppressor STK11 gene, and it plays a vital role in energy metabolism through phosphorylating AMPK at position Thr 172 as a major upstream kinase of AMPK.[36,37] Recent studies have shown that LKB1 exerts a certain anti-inflammatory activity.[38,39] For instance, after the knockout of LKB1, levels of inflammatory cytokines, including TNF-α, IL-1β, major intrinsic proteins 2 (MIP2), and interferon-beta (IFN-β) induced by LPS, significantly increased in bone marrow-derived macrophages.[40] In T cells, LKB1 loss led to decreased AMPK phosphorylation and increased mammalian target of rapamycin complex 1 (mTORC1) activation, leading to T-cell activation and high levels of inflammatory cytokine.[41]

Metformin induces the activation of LKB1. Subsequently, it enhances the LKB1/AMPK pathway, which plays a vital role in various physiopathological processes.[42] In streptozotocin (STZ) and LPS-induced inflammation in mouse lungs,[43] metformin increased the expression of LKB1, phosphor-AMPK, and phosphor-ACC. It markedly decreased the levels of pro-inflammatory cytokines such as TNF-α, IL-lβ, monocyte chemotactic protein-1 (MCP-1), and interleukin-1β (IL-6).

In addition, to investigate whether this protective effect is mediated explicitly via the AMPK pathway, another AMPK activator, AICAR, was introduced into LPS-induced inflammatory peritoneal macrophages under high glucose conditions. It also inhibited the generation of inflammatory cytokines.

Collectively, the aforementioned studies suggest that the LKB1-AMPK-dependent pathway may play a role in mediating metformin's anti-inflammatory effects.

Acetyl-CoA Carboxylase (ACC)

ACC, one of the target molecules of AMPK, is a rate-limiting enzyme in fatty acid synthesis.[44] The AMPK/ACC signaling pathway plays a crucial role in regulating fatty acid oxidation, according to several studies.[45,46]

In TNF-α-induced lipid accumulation in human hepatoma G2 (HepG2) cells, co-treatment with metformin or AICAR decreased the intracellular triglyceride and increased AMPK and ACC phosphorylation.[47] In obese mice, metformin increased levels of phosphorylated AMPK and its downstream target ACC and attenuated pulmonary eosinophilic inflammation.[48] High-fat diets induced metabolic disturbances, renal damage, increased adipokine expression, and macrophage infiltration in mice. Metformin administration exerts protective effects via augmentation of AMPK and ACC activation.[49] Chung et al[50] also verified that metformin alleviated AGE-induced inflammatory responses through AMPK-associated suppression of the inhibitor of kappaB kinase (IKK), NF-κB, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) levels, and it negatively regulated the expression of inflammatory cytokine expression in human neural stem cells (hNSCs), and the transcript and protein expression levels of ACC were also rescued.

Mammalian Target of Rapamycin (mTOR)

mTOR is a serine/threonine protein kinase found in two complexes, mTORC1 and mTORC2. The mTOR signaling pathway is implicated in cell growth, metabolism, energy balance, protein turnover, and autophagy and plays a critical role in inflammatory diseases, autoimmune disorders, and diabetes.[51–53] There is some evidence that metformin inhibits the mTOR pathway both with and without activating AMPK.[54,55] The latter will be discussed in a later section (Figure 1). Metformin inhibits mTORC1 activity dependent on AMPK, tuberous sclerosis complex 2 (TSC2), and AMPK-mediated phosphorylation of Raptor.[56]

Among patients with polycystic ovary syndrome (PCOS), metformin reduced the high levels of TNF-α in B cells. Also, it improved PCOS phenotypes induced by dehydroepiandrosterone (DHEA) in mice through the AMPK/PI3K/mTOR signaling pathway.[57] One recent study by Mohamed et al[58] shows that metformin treatment of diabetic epileptic rats reduced the rise in pro-inflammatory cytokines and apoptotic markers and improved the histopathological results of model rats. Such effects were closely correlated with increased ATP/ADP ratio and reduced death-associated protein (DAP) and mTOR. Besides, a previous clinical experiment demonstrated that metformin had positive effects in reducing inflammation in the colons of people living with HIV (PLWH) undergoing antiretroviral therapy (ART). This effect is achieved through the inhibition of mTOR phosphorylation.[59] Altogether, these suggest the pharmacologic intervention of mTOR may have therapeutic value in diseases characterized by inflammatory responses (Table 1).

NF-κB

NF-κB is a major transcription factor that regulates inflammatory processes, and AMPK modulates NF-κB-dependent inflammation as well.[69,70] In a wide variety of inflammatory disorders, such as atherosclerosis, osteoarthritis, and rheumatoid arthritis (RA), NF-κB is activated.[71,72] Transfection of AMPK α1 siRNA in vascular endothelial cells significantly suppressed AMPK expression and reversed inhibition of NF-κB activation and various adhesion molecules expression induced by vaspin. All these data strongly suggested that AMPK activation is responsible for the inhibition of NF-κB activation.[73]

Activation of the NF-κB signaling pathway contributes to neuronal pathology in response to LPS and cognitive impairments by accumulating inflammatory mediators. It could be achieved through metformin blocking NF-κB pathway.[74] In a study of the streptozotocin-induced-induced diabetic rat models accompanied by evident infiltration of inflammatory cells, phosphor-AMPK was upregulated, and NF-κB p65, as well as the anti-oxidation factors, such as Nrf2, HO-1, and SOD2, were downregulated as compared to model rats not receiving metformin.[75] Notably, no

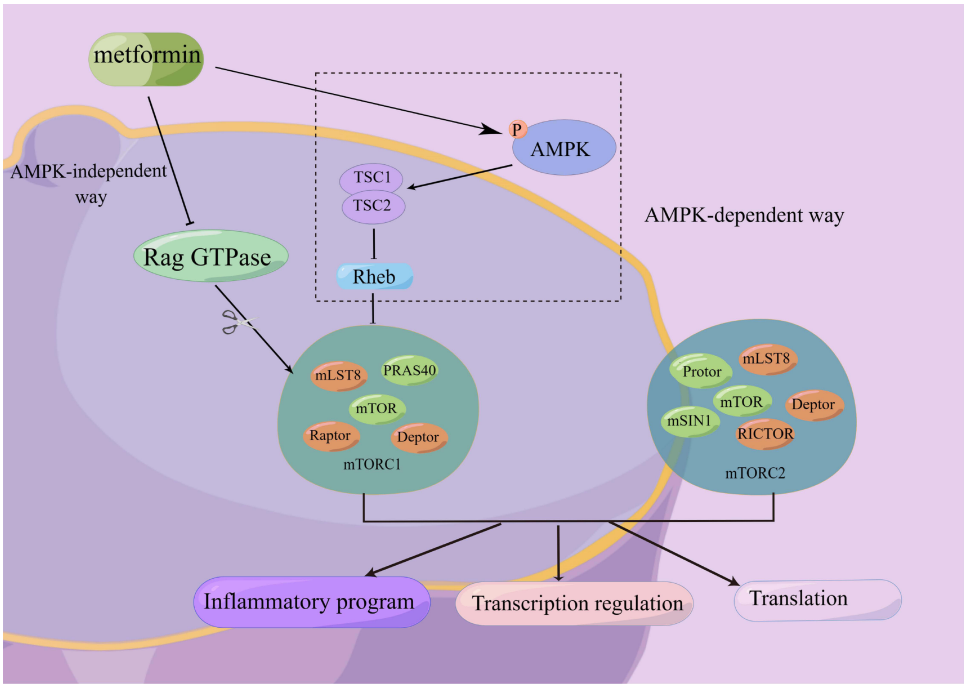

Figure 1 Metformin inhibits mTOR both with and without activating AMPK. There are two ways that metformin could inhibit mTORC1 downstream: (1) After AMPK activity is activated, the TSC1/TSC2 complex (which can inhibit the activity of Rheb) is activated, and then the action of mTORC1 activity is inhibited. (2) Metformin inhibited Rag GTPase activity in an AMPK-independent manner, thereby inhibiting mTORC1. mTOR has two complexes, mTORC1 and mTORC2, which have important regulatory effects on inflammation, gene transcription, protein translation, etc.

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; mTOR, mammalian target of rapamycin; PRAS40, proline-rich Akt substrate 40; TSC, tuberous sclerosis complex; RICTOR, rapamycin-insensitive companion of mTOR; Rheb, ras homolog enriched in the brain; mLST8, mammalian lethal with sec-13 protein 8; Raptor, regulatory associated protein of mTOR; Deptor, DEP domain-containing mTOR-interacting protein; mSIN1, mammalian stress-activated protein kinase (SAPK)-interacting protein 1.

significant drop in blood glucose level was observed after metformin treatment, suggesting that metformin ameliorated meibomian gland dysfunction (MGD) by regulating the AMPK/NF-κB axis instead of reducing blood glucose.

Signal Transducer and Activator of Transcription 3 (STAT3)

A master transcriptional regulator named STAT3 plays an essential role in cell survival, proliferation, differentiation, and inflammatory signaling cascade.[76] Persistent STAT3 activation has been observed in several inflammatory diseases.[77–79] Therefore, the most effective way of targeting STAT3 might be an attractive therapeutic strategy for suppressing the

Table 1 Effects of Metformin Targeting mTOR on Inflammatory Diseases

Disease	Subjects	Pathways	Effects	Reference
Scleroderma	Mice	mTOR-STAT3 signaling	↓IL-6, IL-1β, IL-17, TNF-α, TGF-β, Col1a, Th17 ↑p-AMPK	[60]
Silica-induced pulmonary fibrosis	Human and rats	AMPK-mTOR signaling	↓TGF-β1, TNF-α, IL-1β, α-SMA, p62 ↑E-cadherin, p-AMPK, autophagy	[61]
Thyroid-associated ophthalmopathy	Human	AMPK-mTOR signaling	↓IL6, IL-8, CXCL1, CXCL2, CCL2, HA,α-SMA, ITGA5, COL1A1, COL2A1, COL3A1, FN1, ITGB1, and p-Smad2 ↑Autophagy	[62]
Gout	Human	mTOR signaling	↓The rate of monocyte death, inflammation, and frequency of gout flares ↑Autophagy	[63]

(*Continued*)

Table 1 (Continued).

Disease	Subjects	Pathways	Effects	Reference
Polycystic ovary syndrome	Human and mice	AMPK-PI3K-mTOR signaling	↓TNF-α, mitochondrial membrane potential (MMP), ROS Reverse ovulatory dysfunction, disturbed estrous cycle, and abnormal ovarian morphology, and restore the impaired glucose tolerance	[57]
Psoriasis	HaCaT cells	mTOR signaling	↓Proliferation, p-p70S6K, IL-6, TNF-α, and VEGF ↑Apoptosis	[64]
Liver injury	Rats	mTOR-HIF-1α signaling	↓α-SMA, TIMP-1, hs-CRP, TNF-α, IL-6,ALT and AST	[65]
Spinal cord injury	Rats and microglial cells	AMPK-mTOR signaling	↓Apoptosis, inflammation ↑Axon regeneration, M1 to M2 phenotype polarization, and autophagy	[66]
Intestinal injury	HUVECs, HIECs, and BALB/c mice	mTOR signaling	↓Senescence, intestinal canal inflammation, and oxidative stress	[67]
Sepsis-associated myocardial injury	C57BL/6J mice and H9c2 cells	AMPK-mTOR signaling	↓Cardiac dysfunction, myocardial enzymes, and cardiac hydroncus ↑Autophagy	[68]

inflammatory response and keeping homeostasis. Through STAT3 inhibition and promoting STAT5 signaling, metformin could alleviate T-cell-mediated inflammation by regulating the balance between Th17s and Tregs.[80,81] More specifically, this may be due to STAT3 and STAT5 competing for the same IL-17 promoter binding site.[82] However, another study showed that inflammatory injury during sepsis in mice could not be relieved or even worsened after STAT3 inhibition, and obesity amplified this effect.[76] One possible explanation for the mechanism is the interaction between STAT3 and leptin, which are affected during sepsis. Future studies are required to understand the impact of STAT3 activation on inflammatory diseases. Overall, the current understanding of the mechanism by which metformin regulates T cell function in autoimmune diseases is partly through AMPK/STAT3 signaling.

Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)

An essential transcription factor, Nrf2, is a basic leucine zipper transcription factor.[83] As well documented, Nrf2, a central factor for regulating the antioxidant and cytoprotective gene expression, is indispensable for controlling inflammatory responses.[84,85] The loss of Nrf2 in the skin prolonged the inflammatory response after wounding, and Nrf2-knockout mice were significantly more likely to develop multi-tissue inflammation than normal mice.[86,87] In line with the unequivocal role of the Nrf2-pathway in reactive oxygen species (ROS) detoxifying and inflammation suppression, the potential crosstalk between AMPK and Nrf2 signaling pathways has been noted.[17] A natural antioxidant has been reported to provide therapeutic neuroprotection against inflammation and oxidative stress caused by cerebral ischemia-reperfusion via activating the AMPK/Nrf-2 pathway.[88]

Thus, what is the exact role of the Nrf2-related pathway in metformin-induced effects? In primary bovine mammary epithelial cells (PBMECs), metformin was shown to reduce proinflammatory cytokine secretion. In addition to the anti-inflammatory effect, metformin exhibited antioxidant properties through activation of the Nrf2 pathway, and this activation is significantly AMPK-dependent.[89] Moreover, metformin could dramatically improve the condition of chronic obstructive pulmonary disease (COPD) by reducing the inflammatory response and oxidative stress as well as reducing pathological injury. The molecular mechanism may be related to the Nrf2/HO-1 activation and subsequently activating its downstream target MRP1, then alleviating the glucocorticoid resistance.[90]

AMPK-Independent Pathways

However, several studies suggested that AMPK is not the only pathway for metformin's beneficial anti-inflammation effects.[91,92] Several lines of evidence indicate that some kinases and redox-related transcription factors are regulated by metformin via an AMPKα-independent pathway, including STAT3, mTOR, hypoxia-inducible factor-1α (HIF-1α), and SIRT1.[93–96]

For example, a study performed in murine burn models demonstrated that metformin prevented pathological browning and lipolysis, improving outcomes for burn patients. The reason for this could be metformin's tissue-specific actions on murine and human burn white adipose tissue (WAT), which functioned via an AMPK-independent mechanism through protein phosphatase 2A (PP2A) activation. However, AICAR, another potent AMPK agonist, did not show this effect. In the adipocytes culture experiment, metformin was shown to significantly decrease the phosphorylated c-Jun N-terminal kinase (JNK) p46 and the mRNA levels of TNFα, IL-1β, and IL-6, as well as increased adipocyte expression of PFKFB3/iPFK2. Notably, treatment of adipocytes with metformin did not significantly alter the phosphorylation state of AMPK and failed to suppress the intracellular proinflammatory responses in PFKFB3/iPFK2-knockdown adipocytes effectively.[97] These findings were consistent with a previous study.[98] That is, metformin appears to suppress proinflammatory responses of adipocytes in a manner independent of AMPK.

It is widely recognized that the AMPK-mTOR pathway is indispensable in metformin exerts anti-inflammatory effects. Many reports still show that metformin inhibits inflammation by modulating mTOR independently of the AMPK pathway. Metformin has been reported to directly inhibit mTORC1 activation in a Rag GTPase-dependent manner rather than dependent on TSC1/2 or AMPK. These observations were consistent with two distinct pre-clinical models of cancer and diabetes. Thus, direct inhibition of mTORC1 inhibits inflammatory response.[94] In HepG2 cells transfected with DN-AMPK, metformin was still effective in reducing ROS production, although not to the same extent as in control cells. It indicates metformin may work independently of AMPK as well as through it.[99]

In primary murine bone marrow-derived macrophages (BMDMs), metformin inhibited LPS-induced production of ROS and IL-1β and elevated levels of the anti-inflammatory cytokine IL-10. Furthermore, metformin could also exert a protective effect in AMKPα1- or AMPKβ1-deficient cells. These effects did not depend on AMPK activation and were possibly attributed to the inhibition of reverse electron transport.[100]

Similar effects have been demonstrated in wild-type (WT) and AMPKα2$^{-/-}$ mice exposed to PM2.5 every other day and subsequently received metformin. However, metformin therapy effectively attenuated lung injury and cardiac dysfunction caused by PM2.5 in both the WT and AMPKα$^{2-/-}$ mice. Furthermore, systemic and pulmonary inflammation, pulmonary and myocardial fibrosis, and oxidative stress were reduced in two mice models, suggesting that these effects are AMPK-independent.[101]

Mitochondrial Dysfunction

Mitochondria are the cell's power stations and the source of various pro-inflammatory signals that activate the immune system and generate inflammatory responses. Mitochondrial damage can lead to mitochondrial dysfunction, oxidative stress, inflammation, inhibiting respiration, and decreasing membrane potential.[102] It is also the primary subcellular target of metformin. Multiple studies have demonstrated that metformin can reduce mitochondrial respiration via inhibiting complex I of the electron transport chain, leading to an increase in the AMP/ATP ratio and activating AMPK, thereby alleviating the mitochondrial fragmentation, blocking the production of ROS produced by mitochondria, lessening activation of NLRP3 inflammasomes and the cytoplasmic release of mitochondrial DNA (mtDNA), thus reducing inflammation by downregulating the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1β.[54,100,103,104]

NLRP3

A growing body of evidence indicates that NLRP3 inflammasome regulates the innate immune system and induces inflammatory responses, which consists of caspase adaptor (ASC), pro-caspase-1, and NLRP3 protein, to activate caspase-1 and thus produce high numbers of mature IL-1β.[105,106] Several studies have confirmed that mitochondrial dysfunction and consequent overproduction of ROS are necessary to activate the NLRP3 inflammasome.[107,108]

Yang et al[109] have shown that metformin could inhibit the NLRP3 inflammasome by activating the AMPK/mTOR axis in diabetic mice and high glucose-treated cardiomyocytes, and AMPK inhibitor reversed the upregulation of NLRP3, caspase-1, and IL1-β expression. Besides that, another study also drew a similar conclusion.[30] It reported that metformin inhibited NLRP3 by activating phosphorylated AMPK. At the same time, the expression levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β as well as NLRP3, were decreased when treated with metformin, a special stimulator of NLRP3 (nigericin), abolished the protective effects of metformin in neonatal rat ventricular myocytes. Additional studies have indicated that the NLRP3 inflammasome is an essential inflammatory target in several brain disorders, including depression.[110,111] A recent study found that metformin could act as an antidepressant partly by suppressing peripheral and central NLRP3 inflammasome activation, subsequent caspase-1 cleavage, and interleukin-1β secretion.[112]

Oxidative Stress

Oxidative stress is an imbalance between the formation of oxidative free radicals, also known as reactive oxygen species (ROS), and defense mechanisms by antioxidants.[113] It is well-recognized that inflammation is closely associated with oxidative stress. Oxidative stress can cause the activation of a variety of transcription factors, such as NF-κB, protein-1 activator (AP-1), and hypoxia-inducible factor-1 (HIF-1), leading to the expression of various genes involved in inflammatory pathways.[114–116] The inflammation triggered by constant oxidative stress is the cause of the development of many chronic diseases. During atherosclerosis, oxidative damage increases endothelial permeability, resulting in lipoproteins being released into the subendothelial space and inflammation.[117]

Metformin has been proposed to exert an anti-inflammatory effect through its antioxidant activity.[118] Metformin could suppress the CEBP-homologous protein (CHOP), an endoplasmic reticulum stress (ERS) marker, thus serving cardio-protective roles by attenuating ERS.[119] Araújo et al[120] found that metformin restored the marker of antioxidant genes expression such as malondialdehyde (MDA), a major end product of lipid peroxidation, and superoxide dismutase (SOD) (involved in the neutralization of ROS) in a rat model of periodontitis, implied a strong antioxidant effect, thus caused significant reductions in the expression of pro-inflammatory factors such as IL-1β and TNF-α as well as COX-2 immunostaining. In vitro and in vivo experiments indicated that metformin ameliorated aldo+salt-induced oxidative stress by inhibiting TRAF3IP2 activation, which is an oxidative stress-responsive cytoplasmic adapter molecule, and finally inhibited inflammatory cell infiltration and the pro-inflammatory factors expression such as IL-6, IL-17, and IL-18.[121] Metformin relieved oxidative stress by reducing ICAM1 and COX-2 expression and reducing prostaglandin E2 (PGE2) and endogenous mitochondrial ROS production at μM concentrations, thereby inhibiting subsequent inflammatory signaling and metastatic progression in breast cancer cells.[122] Moreover, a randomized clinical trial indicated that metformin was more efficient in reducing oxidative stress and restoring antioxidant reserves than lifestyle modification alone in patients newly diagnosed with type 2 diabetes.[123]

Nevertheless, numerous studies have suggested that high doses of metformin may compromise mitochondrial function and induce oxidative stress.[124,125] As a case in point, Picone et al[125] observed that ROS production level increased in the human lymphocytes by treatment of metformin at a range of concentrations (5, 10, 20 mM), higher than the regular dose. Hence, different concentrations of metformin application may lead to other, even divergent results. For this reason, concomitant medication should be strictly controlled in future studies.

Mitochondrial DNA (mtDNA)

In mitochondria, extrachromosomal circular DNA is found as mtDNA.[126] In recent years, mtDNA has been recognized as a biomarker of oxidative stress.[127] Increased oxidative stress directly attacks mtDNA, compromising mitochondrial physiology and leading to mitochondrial dysfunction, resulting in an inflammatory response.[128,129] Mutations, deletions, and depletions of mtDNA have been observed in several diseases and pathological disorders.[130,131] The released mtDNA could act as endogenous damage-associated molecular patterns (DAMPs) and induce subsequent inflammation by directly activating the inflammasomes such as NLRP3 and AIM2.[129,132,133] The above research suggested that the accumulation of dysfunctional mitochondria and the release of mtDNA after mitochondria damage play a critical role in the inflammatory response.

Due to the inhibition of mtATP production and the LPS-induced ATP-dependent synthesis of the NLRP3 ligand mtDNA, metformin could reduce Ox-mtDNA generation, thus attenuating acute respiratory distress syndrome (ARDS), which is an inflammatory condition and is associated with high mortality rates.[92] Moreover, metformin treatment profoundly abrogated mtDNA-ATP-induced mitochondrial ROS production, NLRP3 and caspase-1 activation, the release of IL-1β and IL-18 in the RAW264. 7 macrophages.[34]

Immune Cell Populations

Monocytes-Macrophages

Macrophages are well known that play a pivotal role in inflammatory responses. They recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) via pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) to initiate rapid protective immune responses, serving as the first line of defense against invading pathogens.[134,135] However, when macrophages are activated, excessive inflammatory factors are released, aggravating the injured tissues and triggering inflammation.[136]

Metformin has anti-angiogenic and antitumor properties, and these effects have been demonstrated to be closely associated with the suppression of M2-like polarization of tumor-associated macrophages (TAMs).[137] It has been previously shown that in the intestine, the ability of monocytes differentiate into macrophages was inhibited by a supra-pharmacological dose of metformin via AMPK induction, resulting in decreased inflammatory cytokine production. In addition, it activated macrophage polarization into anti-inflammatory phenotypes of M2.[138] It has similarly been shown in ApoE$^{-/-}$ mice as an atherosclerosis model.[35] In addition, metformin has been reported to improve inflammation at a low level in obesity by modulating macrophage polarization to anti-inflammatory M2 type partly via activation of the AMPK pathway.[139] Similar findings have been demonstrated in mice with the multiple sclerosis model autoimmune encephalomyelitis. In addition, another clinical study concerning pancreatic cancer also found that metformin could alter the tumor-educated macrophage polarisation and reduce the tumor cell invasion.[140] Numerous studies have indicated that metformin alleviates inflammatory response in macrophages, generally through AMPK activation, with subsequent inhibition of the production of mitochondrial ROS (mtROS).[141,142] While apart from affecting macrophage polarization, metformin could play an anti-inflammatory role by inhibiting the fatty acid synthesis and Akt palmitoylation in macrophages.[143]

Neutrophils

Neutrophils are critical inflammatory cells in the early inflammatory response of innate immunity. Neutrophils can be preferentially recruited to inflamed sites to exert their essential function.[144] Activated neutrophils could release neutrophil extracellular traps (NETs) (which contain antimicrobial proteins and chromatin) as a form of non-apoptotic cell death termed "NETosis",[145] which is considered to be a host defense mechanism.[146] NETosis not only rap and eliminates diverse pathogens1, but also degrades pro-inflammatory mediators, preventing the inordinate inflammatory response.[147] Indeed, NETosis-impaired individuals exhibit an overshooting inflammatory response.[147] Excessive NETosis is also a hallmark of sepsis and may exert tissue damage and exacerbate pathological conditions.[148,149]

Metformin may alleviate inflammatory diseases with the mechanism by blunting the NETosis has been reported. For example, a randomized controlled trial by Menegazzo et al[150] reported that metformin exerted a protective effect against NETosis to reverse the diabetic inflammatory milieu in vitro and in vivo. In some cases, the production of ROS is required for NETosis.[151] By inhibiting complex I of the mitochondrial electron transport chain, metformin decreases ROS production.[152] A significant role for NETs has been found in systemic lupus erythematosus (SLE) recently, as they promoted plasmacytoid dendritic cells (pDCs) activation and differentiation as well as mediated activation of the pDC-IFN-α pathway, which led to potentiate the autoinflammatory feedback loop.[153,154] Metformin has been found to decrease PMA-induced NETosis, the generation of interferon-alpha (IFN-α), and the NET-mtDNA copies, which are highly pro-inflammatory.[155] Ultimately, metformin reduced disease activity through this treatment and improved survival in SLE patients.[156]

T Cells, B Cells, and Dendritic Cells

Dysregulated immune cells such as T lymphocytes, B lymphocytes, and dendritic cells (DCs) cause autoimmune diseases, which have characteristics of both autoimmune diseases and autoinflammatory diseases.[157]

T cells are crucial in host immune defense and immune-mediated inflammatory diseases.[158,159] Trafficking of T cells from peripheral blood and lymphoid tissues to sites of inflammation is a key regulatory component.[160] The high-fat diet reduced T cell recruitment to the liver, but metformin treatment reversed this effect, and diet-enhanced inflammation was alleviated.[161] Moreover, Th17 cells are a subset of CD4T cells that secrete proinflammatory cytokines, including IL-17.[162] In a mouse model, metformin ameliorated scleroderma by inhibiting Th17 cells through mTOR-STAT3 signaling.[60] Similar findings have been demonstrated in a murine model with acute graft-versus-host disease (aGVHD). Researchers found that metformin could reduce the number of Th1 and Th17 cells and reciprocally upregulate Th2 cells and Treg. The outcomes were achieved via inhibiting the mTOR/STAT3 pathway and inducing autophagy.[163]

Apart from affecting T cells, metformin was also reported to protect women with polycystic ovary syndrome (PCOS) and PCOS mice induced by DHEA via inhibiting TNF-α production in pathological B cells.[57] It was also shown in this study that metformin triggered metabolic reprogramming in B cells of PCOS patients, leading to alterations in mitochondrial morphology, a reduction in mitochondrial membrane potential (MMP), decreased generation of ROS, and reduced glucose uptake. Furthermore, metformin altered metabolic intermediates in splenic B cells from PCOS-model mice.

Accumulating evidence has indicated that autoantibodies derived from abnormal activated B cells contribute to the onset of SLE.[164,165] Interestingly, a study in Roquin$^{san/san}$ mice, a murine model of SLE, showed that metformin inhibited systemic autoimmunity by suppressing B cells differentiation into plasma cells and decreasing the number and size of germinal centers.[166] Similar results were obtained in murine Sjögren's syndrome.[13]

Dendritic cells (DCs) are the most efficient antigen-presenting cells (APCs) that can initiate and regulate T cells, B cells, and natural killer cells, leading to an adaptive immune response.[167] Metformin has been demonstrated to reduce major histocompatibility complex (MHC) class I- and class II-restricted presentation of ovalbumin by decreasing co-stimulatory molecule expression.[168]

Gut Microbiota

The gut microbiota is a complex microbial community consisting of more than 100 trillion microorganisms in the gastrointestinal tract of humans, including bacteria, viruses, fungi, and protozoa.[169] Mounting studies have demonstrated that the alteration in gut microbiota composition has been correlated not only with the intestinal inflammatory disorder but also with numerous diseases characterized by systemic chronic inflammation and metabolic disorders,[170] such as allergy, asthma, obesity, and atherosclerosis.[171–175] The induction of inflammatory processes perhaps linked to the translocation of microbial products into circulation.[176] Recently, Yan et al[177] discovered that the bacteria proportions were closely related to inflammatory indicators. The result was that the relative abundance of Firmicutes was positively correlated with TNF-α, whereas Bacteroidetes was inversely correlated with plasma TNF-α, aorta IL-1β, and IL-6. Moreover, the abundance of Proteobacteria was positively correlated with the levels of plasma TNF-α, IL-6, and aorta IL-6. Akkermansia was negatively related to plasma LPS and aorta IL-6. Similarly, a negative correlation existed between Bifidobacterium and plasma LPS, TNF-α, and aorta IL-1β. In contrast, a positive correlation existed between Romboutsia and plasma indicators of inflammation such as IL-6, IL-1β, and TNF-α resistance. Alternatively, the study revealed that the primary reason for the effectiveness of metformin treatment in anti-atherosclerosis might be alleviating macrophage-mediated inflammation via rectification of gut dysbiosis.

Chronic low-grade inflammatory response involves metabolites such as endotoxin derived from the gut flora.[178] It has been discovered that non-alcoholic fatty liver disease (NAFLD), which includes a variety of pathological changes such as no-alcoholic steatohepatitis (NASH), is linked to an increase in gut permeability that is associated with increased levels of small intestine bacterial overgrowth in humans.[179] Along a similar line, endogenous gut-derived bacterial endotoxin is considered a vital cofactor mediating the pathogenesis of NASH.[180,181] Thus, the primary source of circulating endotoxin among nonseptic individuals is bacterial endotoxin translocation through a weakened gut barrier. In one study, mice's fat-

, fructose- and cholesterol-rich diet (FFC) feeding were used to model NAFLD.[182] The mice were fed a metformin diet for six weeks, and the markers of inflammation, liver health, intestinal barrier function, and microbiota composition were analyzed. The markers of the inflammation and lipid peroxidation were significantly decreased following metformin treatment. Compared to the NAFLD mice model, intestinal microbiota composition in the proximal small intestine was also changed in metformin-fed mice. These data confirm that metformin exerts anti-inflammatory effects partly by regulating gut microbiota function.

In a dextran sulfate sodium-induced ulcerative colitis (UC) murine model, metformin treatment exerted anti-inflammatory and mucus-protective effects by changing the gut microbiota by increasing the abundance of Akkermansia muciniphila and Lactobacillus, both of which possess anti-inflammatory property.[183] Additionally, a clinical study confirmed that metformin could regulate the gut microbiota in patients with type 2 diabetes, which may explain the beneficial pleiotropic effects of metformin.[184] Moreover, AMPKα1 activation by metformin has been shown to counteract the effect of the intestinal barrier dysfunction in promoting inflammation which was triggered by C-Jun N-terminal kinase (JNK).[185]

Autophagy

Autophagy is a multi-step, intracellular lysosomal degradation pathway that degrades or removes damaged proteins and organelles in the cytoplasm.[186] Thus, steady-state autophagy is essential for preserving cell homeostasis, while additional autophagy induced by environmental stress may be beneficial for protecting cells.[187,188] Some studies have shown that inflammation is tightly related to autophagy, and enhanced autophagy can regulate inflammatory reaction.[189]

Autophagy can be categorized into macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Among these, macroautophagy (hereafter autophagy), mediated by organelles known as autophagosomes, is the most well-studied and most relevant to inflammasome studies.[190,191] Most reported inhibitory effects on inflammation have been associated with macroautophagy, while the implications of other types of autophagy remain unclear.[192] The multi-step process of autophagy requires the collaboration of a variety of autophagic proteins. So far, over 30 autophagy-related proteins (ATGs) are involved in autophagy regulation.[193] Among these, microtubule-associated protein light chain 3 (LC3), Beclin1, and autophagy-degrading substrate SQSTM1/p62 are the most widely studied autophagy-related proteins.[194] It has been suggested that autophagy-related proteins regulate NALP3-dependent inflammation by maintaining mitochondrial integrity.[195] Mechanistically, autophagy could decrease NLRP3 activation via diverse mechanisms, including increasing the p-AMPK levels[196] and suppressing the mTOR signaling pathway,[197] and continuously clearing various endogenous inflammasome agonists and components, such as ROS and damaged mitochondria.[107,198] Kabat et al[199] also found that selective deletion of Atg16l1 in T cells in mice resulted in increased pro-inflammatory cytokine production in the gut. As a result, the mice developed spontaneous intestinal inflammation and secreted antibodies against gut bacteria and food, indicating the critical role of autophagy in suppressing intestinal inflammation.

Pharmacological modulation of autophagy is an attractive therapeutic target against inflammatory diseases with metformin treatment (Figure 2). In a Kawasaki disease (KD) vasculitis model, cardiovascular lesions were associated with activation of NLRP3, high ROS levels, and increased systemic 8-OHdG release. Damage induced by ROS and impaired autophagy/mitophagy deteriorated the development of murine KD. In this study, metformin treatment relieved inflammatory response from lactobacillus casei cell wall extract (LCWE), reduced cardiovascular lesions, and enhanced autophagic flux significantly, indicating its potential in treating inflammation diseases. These effects were realized by metformin reducing ROS and activating autophagy through the AMPK axis.[200]

The expression of p-AMPK and the autophagy-related protein LC3 II and p62 were upregulated, and mTOR levels were downregulated by metformin treatment.[34] These changes were reversed after treatment with AMPK inhibitor Compound C. What's more, using the autophagy inhibitors chloroquine (CQ) and 3-methyladenine (3-MA) and knocking down or knocking out Atg5 (autophagy-related gene 5) led to the counteraction of the inhibitory effects of metformin on NLRP3 inflammasome activation induced by hydrogen peroxide and mtDNA-ATP, as well as the release of IL-1β, IL-18 and ROS production in RAW264. 7 macrophages.[34]

Autophagy has a crucial role in cardiovascular homeostasis.[201] Accumulating evidence indicated that inflammatory mediators could be therapeutic targets for heart failure treatment.[202,203] Further, a growing body of research suggests that

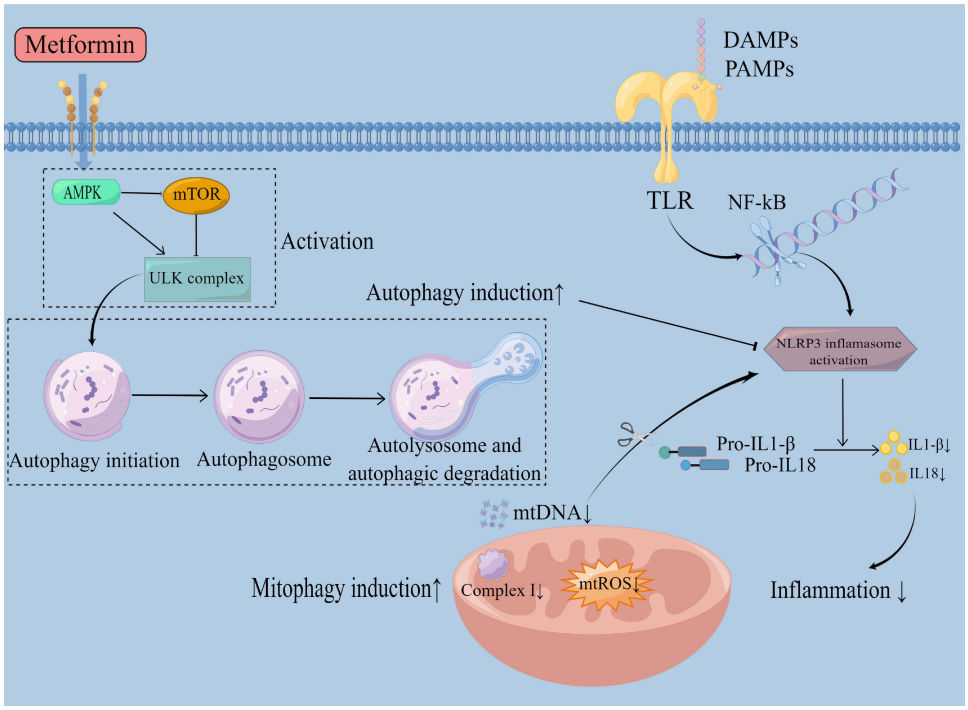

Figure 2 Metformin reduces inflammation by regulating the autophagy process. TLR family members activated by PAMPs or DAMPs initiate the innate immune response as a response to infection or injury. Then, it activates NF-kB and further activates the NLRP3 inflammasome. Upon NLRP3 activation, pro-IL1-β and pro-IL18 were cleaved into mature and active IL1-β and IL18. These cytokines are subsequently released, initiating an inflammatory response. Metformin activates AMPK, inhibits mTOR, and activates the ULK complex, thus inducing autophagy and further blocking the activation of NLRP3, inhibiting inflammation. Autophagy involves the formation of autophagosomes, fusion of autophagosomes with lysosomes, and degradation of the autophagy-lysosomes. Metformin could also scavenge mtDNA and mtROS through mitophagy induction via the inhibition of mitochondrial complex I, inhibiting NLRP3 inflammasome activation, thus eventually inhibiting inflammation.

metformin exerts cardiovascular protection irrespective of glucose change and associated effects.[109,204–206] Metformin has been reported to regulate autophagy in various cardiovascular diseases, including heart failure, I/R injury, and diabetic cardiomyopathy. However, it has been demonstrated that autophagy activated by I/R appears to act as a compensatory protective mechanism for I/R injury.[207]

Interestingly, there were almost opposite conclusions about autophagy's value in I/R injury. A previous study reported that the metformin-driven cardioprotection function involves a decreased level of autophagy and restores the impairment of autophagosome processing, ultimately reducing myocardial inflammation. The study further indicated that metformin's beneficial effect was mediated by down-regulating the autophagy caused by the Akt signaling pathway.[208] Obviously, there is still controversy regarding the role of autophagy in I/R injury.[209–211]

Of note, over-induction of autophagy leads to inflammatory increases have also been reported. A recent study on periodontal ligament stem cells (PDLSCs) suggested that force-stimulated PDLSC autophagy differentiates macrophages into the M1 phenotype by suppressing the AKT signaling pathway, aiding the inflammatory bone remodeling and tooth movement process.[212] Besides, another study indicated that ER stress-induced autophagy may exacerbate the severity of inflammatory bowel diseases.[213] Moreover, a recent randomized controlled clinical study found that inhibiting macrophage autophagy by combining corticosteroids and statins enhanced IL-10 production, thereby controlling asthma inflammation.[214] Therefore, autophagy is a "double-edged sword" in inflammation.[215] Metformin has been shown to down-regulate autophagy by reducing glutamine metabolism and ammonia accumulation at micromolar concentrations.[216]

However, most of the literature focused on the promoting effect of metformin in autophagy. There are studies indicating otherwise. Whatever the case, metformin's effect on autophagy activation could be dose-dependent and cell-type specific. More research is needed to elucidate the role of metformin in autophagy further.

Aging

Aging is often accompanied by a state of chronic, low-grade systemic inflammation, which is a major risk factor associated with high levels of mortality and morbidity.[217] Many age-related diseases, such as atherosclerosis,[218,219] rheumatoid arthritis,[220] and Alzheimer's disease,[221] are associated with senescence-associated inflammation caused by pro-inflammatory molecules. Senescent cells occur and accumulate in-vivo, which could increase the production of pro-inflammatory cytokines, including TNFα and IL-6, and matrix metalloproteins, as well as angiogenic factors, creating a secretory profile called Senescence-Associated Secretory Phenotype (SASP), which could result in immune system dysregulation and induce inflammation and senescence.[222–225] Therefore, treatment strategies can target cellular senescence to relieve inflammation.

Accumulating evidence demonstrates an anti-inflammatory role of metformin in aging-related inflammation via removing senescent cells and regulating the SASP. Metformin was shown to trigger a DNA damage response that induced senescent cells' immune clearance via the activation of ATM.[226,227] Moreover, mechanisms that metformin accelerate self-renewal include autophagy and prevent several pathologic and destructive changes. In many senescence models, metformin alleviated cellular senescence in a DICER1-dependent mechanism. It reduced the expression of the p16 and p21 proteins and the content of the inflammatory cytokines that are hallmarks of the SASP.[228] Similarly, it has been shown that the effects of metformin on preterm birth due to LPS could be alleviated through slowing premature decidual senescence that led to the loss of placenta/fetus attachment to the maternal decidua.[229] Furthermore, a randomized controlled trial by Long et al[230] demonstrated that metformin could enhance the response of older adults to resistance exercise training by alleviating inflammation in muscle tissue, highlighting the role of metformin in inhibiting aging-related inflammation.

Although studies have shown that elderly patients, especially those with inflammatory diseases, appear less sensitive to metformin, there are some additional benefits. Additional studies are needed to determine whether metformin could reduce inflammation and relieve symptoms of inflammation-associated diseases by reversing the aging processes.

Conclusion

Metformin has been used as an anti-diabetic drug for decades.[231] These years, abundant clinical and basic studies have shed light on various critical roles of metformin in various inflammatory diseases. Most studies have focused on targeting AMPK and inhibiting mitochondrial ETC complex I.[232] There are still many other possible mechanisms to investigate. Here, we summarize the latest research on the potential mechanisms of metformin in different inflammatory disorders. Gaining more insight into how metformin regulates inflammatory responses and characterizing the exact targets may be helpful in personalized therapy and the clinical use of metformin. The specific role and precise mechanisms of metformin under different physiological or pathological conditions need further study, and these efforts should be the focus of future research.

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Disclosure

The authors report no conflicts of interest related to this work.

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