Review Article

Role of Butylphthalide in Immunity and Inflammation: Butylphthalide May Be a Potential Therapy for Anti-Inflammation and Immunoregulation

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Inflammation and immunity play an essential role in disease pathogenesis. 3-N-Butylphthalide (NBP), a group of compounds extracted from seeds of *Apium graveolens* (Chinese celery), has been demonstrated as an efficient and effective therapy for ischemic stroke. The amount of research on NBP protective effect is increasing at pace, such as microcircular reconstruction, alleviating inflammation, ameliorating brain edema and blood-brain barrier (BBB) damage, mitochondrial function protection, antiplatelet aggregation, antithrombosis, decreasing oxidative damage, and reducing neural cell apoptosis. There has been increasing research emphasizing the association between NBP and immunity and inflammation in the past few years. Hence, it is aimed at reviewing the related literature and summarizing the underlying anti-inflammatory and immunoregulatory function of NBP in various disorders.

1. Introduction

There is growing evidence that inflammation and immune response are critically involved in the initiation and severity of a series of other significant diseases. Neurological disorders, for example, acute ischemic stroke, activate inflammatory and immune cells within the central nervous systems (CNS) and induce the infiltration and accumulation of the inflammatory and immune cells from the peripheral system, which exacerbates the pathologies and worsens neurological prognosis [1]. Thus, the treatments with the ability to modulate inflammatory and immune responses can effectively prevent disease progression and minimize related disabilities.

3-N-Butylphthalide (NBP) is composed of optical isomers l-3-N-butylphthalide (l-NBP) isolated from seeds of *Apium graveolens* (Chinese celery), d-3-N-butylphthalide (d-NBP), and a synthesized compound, dl-3-N-butylphthalide (dl-NBP). NBP is oxidized by cytochrome P450 (P450) after oral administration. Moreover, hydroxylation of the n-butyl side chain and C-3 are involved in the primary metabolism process. Then, dl-NBP converts to four principal metabolites, including 10-keto-NBP (M2), 3-hydroxy-NBP (M3-1), 10-hydroxy-NBP (M3-2), and NBP-11-oic acid (M5-2), and finally is discharged mainly by the kidneys [2].

Furthermore, dl-NBP has been approved by the State Food and Drug Administration of China and introduced in the Chinese market as an anti-ischemic drug since 2002. Former clinical research and animal experiment concerning ischemic stroke have demonstrated that dl-NBP has multiple functions, including microcircular reconstruction [3], alleviating inflammation [4], ameliorating blood-brain barrier (BBB) damage [5], mitochondrial function protection [6, 7], antiplatelet aggregation, antithrombosis [8], decreasing oxidative damage [9], and reducing neural cell apoptosis [10]. Besides, NBP is an efficient and effective therapy for neurodegenerative diseases, brain edema, neural trauma, neurotoxicity, epilepsy, autoimmune diseases, and other nonneurologic conditions [11, 12].
2. The Potential Mechanism of NBP in Immune and Inflammatory Modulation

Some emerging experiments consider NBP as a novel agent for autoimmune disease treatment, such as idiopathic inflammatory myopathies (IIM) [13, 14] and multiple sclerosis (MS) [15]. Additionally, according to the current reports, a shift of macrophage/microglia polarization from proinflammatory M1 to anti-inflammatory M2 phenotype was observed in NBP-dependent ways [16, 17], indicating the possible underlying NBP’s mechanism of inflammatory modulation and the potential of immune regulation. More explorations about the pathways via which NBP exerts a protective role are required. Therefore, based on existing evidence, we review and discuss the possible and potential NBP-mediated functions regarding regulating immunity and inflammation. The utility of NBP, targeting the signal molecules concerning the response to immune and inflammation, can be a promising therapeutic drug for inflammatory and immune-mediated diseases.

2.1. NBP and NF-κB. Nuclear factor-kappa light chain enhancer of activated B cells (NF-κB), as a family of evolutionarily conserved transcription factors, is well known in gene induction in a wide range of biological processes, including neurodegeneration, regulating cell growth and survival to immunity and inflammation [18–21]. Generally, the NF-κB family of transcription factors consists of five members, p50, p52, p65 (RelA), c-Rel, and RelB. In resting cells, inhibitory protein IκBα binds with NF-κB, and the formation of complexes keeps NF-κB as an inactive state in the cytoplasm. In canonical NF-κB signaling pathways, phosphorylation, ubiquitination, and degradation of IκBα in the proteasome then allow NF-κB to translocate to the nucleus, bind to specific DNA binding sites, and initiate the transcription of target proinflammatory genes, upon stimulation such as lipopolysaccharide (LPS) [22, 23]. NF-κB’s specific binding regions have been identified in proinflammatory genes such as TNF-α, IL-1β, and IL-6. Accordingly, targeting the NF-κB pathway is regarded as a therapeutic strategy against inflammatory disorders.

Accumulating research has shown that NBP modulates NF-κB pathways. In current studies, NBP vastly reduced the NF-κB protein level to alleviate inflammation in diverse animal models [24–27] and played a neuroprotective role in demyelination reduced by chronic cerebral hyperperfusion (CCH) [28] and ethidium bromide [29]. Numerous articles have demonstrated that the inactivation of the NF-κB pathway is related to proinflammatory reactions in macrophages. Compared with the control group, LPS-stimulated RAW 264.7 macrophages have a higher protein concentration of NF-κB-related proteins (p65 in the cell nucleus, phosphorylated-IκB-α, phosphorylated-IKK-α/β) and lower cell cytosol protein concentration of p65, confirming that LPS induces the translocation of NF-κB dimers from the cytosol to the nucleus to regulate macrophage/microglia properties. Conversely, pretreatment with AAL, a medicine extracted from Chinese medicinal plant, effectively inhibited this translocation and at the same time reduced production of TNF-α and IL-6 [30]. Similarly, previous studies revealed that administrations of NBP in rat models with cerebral ischemia reperfusion-induced brain injury [31] and spinal cord injury [32] inhibited the expression of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α, via reducing expression of TLR4 and NF-κB (including p-NF-κB, p-IκB-α, and p-IKK-α). Furthermore, the polarization of macrophage/microglia is under control by NF-κB. It has been reported that blocking NF-κB on ovarian cancer cell conditioned media suppressed M1 macrophage-induced metastatic potential [33]. Another study showed that NBP remarkably suppressed the expression of nuclear p65 and reduced proinflammatory molecules in LPS-stimulated as well as MPPC-stimulated BV2 cells by Western blot analysis of nuclear and cytoplasmic fractions. Moreover, NBP have prevented the accumulation of nuclear p65 in response to LPS stimuli by immunofluorescence assay [34]. Based on the above results, we propose that NBP can regulate the polarization of macrophage/microglia via NF-κB.

Besides the relationship with macrophage/microglia polarization, NF-κB is associated with other immune cells. The functions of dendritic cells (DCs) depend on their maturation level. The maturation of DCs, in terms of upregulation of major histocompatibility complex and costimulatory molecules, is under control by activation of the NF-κB pathway, especially the NF-κB protein RelB [35, 36]. Inhibition of NF-κB enables DC to induce Treg formation and Th2 polarization in vitro and in vivo [37, 38]. The indispensable roles of NF-κB proteins in B cell development, maintenance, and function have been demonstrated [39]. Consequently, it has been presumed that NBP’s function on innate or adaptive immunity cells is mediated through NF-κB.

2.2. NBP and p38MAPK. The p38 mitogen-activated protein kinase (p38MAPK, termed here p38) is a vital signaling protein kinase that guides a signaling cascade to transmit extracellular signals to their intracellular targets. Abnormal activity and dysregulation of p38 have been shown to participate in the induction of pathologies such as inflammation [40], cancer [41], autoimmune diseases [42], Parkinson’s disease [41], Alzheimer’s disease [43], cardiac hypertrophy [44], and diabetes [45]. In many cases, p38 regulates inflammation and immunity, which contributes to the development of the diseases.

Some experimental results showed that NBP could regulate p38 expression. In the LPS-induced mouse model of Parkinson’s disease (PD) [46] and rats of cerebral ischemia-reperfusion injury [5], phosphorylated-p38/p38 was significantly reduced following treatment with NBP. Contrary to the previous studies, NBP treatment promoted phosphorylated-p38/p38 in spinal cord injury (SCI) mice and BV2 cells [16]. Furthermore, there is a close association between macrophage/microglia polarization and p38 phosphorylation. For example, the p38 pathway is involved in microglia activation and positively affects microglia’s proinflammatory secretory function in vivo and in vitro [46–49]. Another study showed that Gr-1(+) CD115(+) monocytes in tumor-bearing mice exhibited M2 characteristics. Conversely, LPS could transfer M2-type cells into M1 type through activating the P38 MAPK
pathway, which, in turn, leads to the inhibition of the anti-inflammatory function of Gr-1(+) CD115(+) [50]. In BV2 cells and SCI mice, SB203580, a selective p38 pathway inhibitor, reversed the effect of NBP on inhibition of M1 marker expression and promotion of M2 marker expression [16], implying that NBP can enhance M2 polarization and inhibit M1 polarization in a p38-dependent way. Besides, p38 plays a significant role in macrophages to regulate the activity of transcription factors involved in inflammation response. Macrophages isolated from p38 \( \gamma/\delta \) deficiency mice had lower reduced production of TNF-\( \alpha \), IL-1\( \beta \), and IL-10, which demonstrated that p38 \( \gamma/\delta \) are critical regulatory components of the innate immune response [51]. Environmental and cellular stresses stimulate p38 phosphorylation in macrophages [52], leading to the release of proinflammatory mediators, such as IL-1\( \beta \), TNF-\( \alpha \), PGE2, and IL-12, as well as COX-2, IL-8, IL-6, IL-3, IL-2, and IL-1 from macrophages [53–56]. Inhibition of p38 by its specific inhibitor SB203580 significantly inhibited morphine-induced apoptosis and caspase-3 activation in BV2 cells [57]. Thus, it is reasonable to presume that NBP can regulate the p38 pathway somehow to influence macrophage/microglia activation, polarization, and subsequent expression of inflammatory mediators.

2.3. NBP and HIF-1\( \alpha \). Hypoxia-induced factor (HIF) is a transcription factor consisting of an alpha and beta subunit. There are three known alpha subunits (HIF-1\( \alpha \), HIF-2\( \alpha \), and HIF-3\( \alpha \)) and three beta subunits (HIF-1\( \beta \), HIF-2\( \beta \), and HIF-3\( \beta \), also known as ARNT1, ARNT2, and ARNT3). Except for the contributions to the cells’ ability to adapt to changes in oxygen levels, angiogenesis, cell survival, invasion, and metastasis of the tumor, HIF is also related to the modulation of various immune cells, including macrophages, DCs, neutrophils, and T/B cells [58].

Researchers reported that dl-NBP treatment in a photochemical reaction-induced focal permanent middle cerebral artery occlusion (MCACO) model upregulated expressions of HIF-1\( \alpha \) and VEGF [7]. The expression of HIF-1\( \alpha \) was increased under chronic intermittent hypoxia hypercapnia (CHIH) exposure and was further expressed in rats with chronic NBP administration, which was consistent with the expression of Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (Bnip3), a known HIF-1\( \alpha \) target protein [59]. HIF-1\( \alpha \) expression in the nucleus was extraordinarily increased after rat brain microvessel endothelial cells (BMECs) were exposed to 2-hour oxygen-glucose deprivation (OGD) and 24-hour reperfusion and with NBP treatments [60, 61]. The above results suggest that NBP affects the expression of HIF-1\( \alpha \) to a certain extent.

It is known that HIF-1\( \alpha \) is involved in immune and inflammatory processes. In in vitro and in vivo inflammatory models, the deletion of HIF-1\( \alpha \) dramatically decreased ATP levels in macrophages as well as reduced aggregation, motility, and macrophages’ bacterial killing [62]. Overexpression of HIF-1\( \alpha \) in macrophages promotes M1 polarization with a hyperinflammatory state [63–65], which is via upregulating expression of glycolysis and pentose phosphate pathway intermediates [64]. HIF-1\( \alpha \) leads to T cells differentiating into Th17 through direct transcriptional activation of ROR\( \gamma \)t and subsequently p300 recruitment to the IL-17 promoter. Concurrently, HIF-1\( \alpha \) reduces Treg development by binding with Foxp3 and targeting it for proteasomal degradation [66]. Similar proinflammatory characteristics for HIF-1\( \alpha \) have been revealed in cell metabolism, differentiation, migration, and cell survival of DCs [67, 68], neutrophils [69, 70], and other immune cells [71]. It is reported that HIF-1\( \alpha \)-dependent regulation of NF-\( \kappa \)B is directly involved in regulating neutrophil survival in hypoxia via a comparison between HIF-1\( \alpha \) wild-type and gene knockdown murine neutrophils [69].

However, there are some controversies. NBP alleviates inflammation while it also inhibits HIF-1\( \alpha \) with proinflammatory properties. On the one hand, the role of HIF-1\( \alpha \) produced is different during various stages of the disease. Knockdown of HIF-1\( \alpha \) during the early stage of Mycobacterium tuberculosis (MTB) infection resulted in a heightened disease state in these mice, while blocking HIF-1\( \alpha \) during the late stage of MTB increased macrophage apoptosis and decreased bacillary loads [72]. The same results could be observed in sepsis [73]. Therefore, increasing expression of HIF-1\( \alpha \) induced by NBP has a positive effect during the early stage of immune-mediated diseases. Accordingly, the data suggests that NBP may modulate immune cells (e.g., macrophage and neutrophil) and respond to inflammation through HIF-1\( \alpha \).

2.4. NBP and AMPK/SIRT1. AMP-activated protein kinase (AMPK), a serine/threonine kinase, is considered a metabolic sensor that maintains energy balance at the cellular and systemic levels [74]. Sirtuins (SIRT), as AMPK downstream molecules, belong to the class III histone deacetylase family and are divided into seven subtypes in mammals characterized by the same c.275-amino-acid core deacetylase domain and various N- and C-terminal domains [75]. As the most studied SIRT in mammals, SIRT1 is another nutrient sensor with widespread effects on metabolism and inflammation [76].

Many studies suggest that NBP affects SIRT1 expression. Min et al. confirmed that the neuroprotective effect of NBP under CIHH conditions might be caused by activating the SIRT1/PGC-1\( \alpha \) signaling pathway [59]. At two weeks and four weeks after bilateral common carotid artery occlusion (2VO), NBP treatment suppressed inflammation, reduces demyelination, and promotes oligodendrocyte regeneration by reversing declining levels of AMPK/SIRT1 in CCH rats [28]. The expression of AMPK increased in the model of ischemic stroke after treatment with NBP [77].

Firstly, there is some evidence elucidating the role of AMPK in regulating immune cell metabolism and function. CD8+ T cells with deletion of AMPK\( \alpha \)1 cannot revert to memory cells in metabolic dormancy [78]. Th cell development in response to infection requires AMPK\( \alpha \)1 [79], which is in keeping with the experiments where the upregulation of AMPK increases the number of Treg cells for anti-inflammation [80]. Metabolically regulating immunity and inflammation of AMPK in natural killer (NK) cells was confirmed by the recent emerging report [81]. Pharmaceutical activation of AMPK, as a promising therapy, reduces the
secretion of inflammatory markers (e.g., COX-2 and IL-1) [82, 83]. It is suggested that NBP can affect the differentiation of T cells and the functions of NK cells and finally regulate inflammation and immunity due to its similar effect on AMPK.

Secondly, inhibition of AMPK blocks autophagy via increasing mitochondrial reactive oxygen species (ROS) production, which is a way to reduce inflammation [84, 85]. Further, NBP pretreatment reduces the proinflammatory molecules and prevents oxidative damage by inhibiting the release of NO and ROS production in BV-2 cells [34], in SH-SY5Y neuroblastoma cells [86] and in vitro animal models [5, 77, 87, 88], though increasing AMPK expression [77]. Thus, we speculate that NBP increases activation of AMPK and subsequently influences the expression of NO and ROS from immune cells to regulate inflammation.

Thirdly, the SIRT1-HIF1α axis guides the cytokines’ production from the dendritic cell in the metabolically dependent ways, promotes the differentiation of CD4+ T cells, determines macrophage phenotype, and switches innate immune signals to adaptive immune responses [89, 90], which implies NBP can also produce the same immunomodulatory effect through the SIRT1-HIF1α axis.

Collectively, it indicates that NBP increases the expression of SIRT1 and AMPK to regulate immune and inflammation.

2.5. NBP and PI3K/Akt. Since the discovery of protein kinase B (PKB, also known as Akt) 25 years ago [91] and the identification of phosphatidylinositol 3-kinases (PI3K) as its upstream regulator [92], PI3K/Akt acts as a central node of many signaling pathways, such as immune modulation, tumor cell proliferation, and apoptosis.

Various experiments have shown that NBP affects the PI3K and Akt to play a protective role. P-Akt levels were decreased in the CCH 8-week group but activated in the NBP-treated group, coinciding with the results that NBP activated PI3K/Akt in oxygen-glucose deprivation/reperfusion (OGD/R) [93], the animal model of depression [94], MCAO rats [31], and bone marrow stem cells (BMSCs) [95]. Noticeably, expression of p-Akt instead of total Akt is dramatically increased after NBP treatments. [96]. Similarly, in the study about OGD/R inducing cognitive impairment, there was no significant upregulated these genes [77]. The mRNA and protein expression of Nrf-2 and HO-1 in the NBP treatment group was dramatically increased at 24 hours after cerebral infarction compared with that in the control group [110], which was consistent with the effect of the NBP in a mouse model of amyotrophic lateral sclerosis [25]. Previous studies have shown that NBP increased Nrf2/HO-1 to inhibit atrial structural remodeling and finally prevent atrial fibrillation in heart failure rats [111].

Nrf2/HO-1 participates in regulating inflammatory immune cells through various mechanisms. As the primary anti-inflammatory and antioxidant enzymes are regulated by Nrf2 activation [112], HO-1 expression can affect the switch macrophages to M2 type in vitro [113, 114]. This role of HO-1 in regulating macrophage polarization has also been shown in experimental animal models of diabetes, Crohn’s disease, hypertension, alcoholic liver disease, and bowel damage [114]. Moreover, pharmacologic induction of HO-1 inhibits human Th (T helper) and CD8+ cytotoxic T (TC) cell activation [115] and Treg cell function [116]. In keeping with these observations, biliverdin/bilirubin and CO (production of HO-1) inhibit Th cell activation [115, 117], induce apoptosis in Jurkat T cells [118], and suppress T cell-driven inflammatory pathologies, the rejection of transplanted organs [33], or autoimmune neuroinflammation [117]. Salutary effects of HO-1 are also exerted via sustaining...
tissue function and preventing endogenous proinflammatory ligands released from injured cells causing unfettered immune activation.

Accordingly, it is assumed that the protective effects of NBP are mediated essentially through immunoregulatory effects of Nrf-2/HO-1 exerted in cells of the innate or adaptive immune system.

2.7. NBP and Antioxidative Stress. Many immune-mediated inflammatory diseases are related to free radicals, which results in a high level of cellular oxidative stress and tissue injury. The death of cells driven by overreactive oxidative stress releases their intracellular components. These components act as proinflammatory and immunogenic agonists recognized by pattern recognition receptors (PRRs) expressed in immune cells, such as Mø and DCs [119]. In addition, oxidative-stress-dependent activation of transcription factors modulates the biosynthesis of antioxidant proteins and proinflammatory factors (such as NF-κB and Nrf2 that could be regulated by NBP discussed above), and the activation is associated with inflammation and immune cells [120, 121]. Therefore, we presume the effect of NBP on modulating immune and inflammation might at least in part, be exerted in such ways.

On the one hand, ROS is detrimental to cell structure by interacting with proteins and nucleic acids, especially lipids, resulting in the peroxidation of membrane phospholipids [122]. Severe metabolic disturbances and cell death happen as a consequence [123]. Superoxide dismutase (SOD) is a crucial antioxidative enzyme [124], while increased malondialdehyde (MDA) indicates oxidative damage of membranes, acting as an oxidative stress marker. NBP is shown to regulate the expression of these oxidative and antioxidant markers. The NBP treatment group has higher levels of both SOD and catalase (CAT), and lower levels of both MDA and proinflammatory cytokine (IL-1β and IL-6) were found in major depressive disorder (MDD) rats [26], diabetic rats [125], EAM guinea pigs [14], and BMSCs [95]. In cerebral ischemia-reperfusion injury rats, NBP reduced infarction area, cell apoptosis, blood-brain barrier destruction, and edema content through inhibition of ROS and MDA and via increasing activation of SOD [5]. Moreover, Nrf2 also plays an integral role in the antioxidative stress systems of cells. After being activated by oxidative stress, Nrf2 is transferred to the nucleus, and it binds to the ARE, finally elevating the expression of antioxidant genes and protecting cells from oxidative damage [126]. It has been shown that NBP could increase Nrf2 while reducing cellular oxidative stress. In the same study concerning MDD, researchers found the significantly upregulated level of Nrf2 in the nucleus, and the increasing trend of HO-1 and NQO-1, the Nrf2-downstream antioxidant genes, in NBP treatment [26], consistent with results from the experiment about OGD under NBP administration [77]. Thereby, these experiments suggest that NBP possesses the effect of antioxidation.

On the other hand, the average ROS/RNS, mainly produced by normal mitochondria, functions in healthy tissues to maintain normal physiological activities. Nevertheless, mitochondrial dysfunction may contribute to excessive and deregulated production of these molecules. Improper ROS, in turn, destroys mitochondrial inner membrane integrity, promotes mitochondrial depolarization, stems mitochondrial electron transfer chain, increases the opening of the mitochondrial permeability transition pore, and loses the intracellular calcium homeostasis [127–129]. It becomes a vicious circle and finally leads to intracellular oxidative stress and tissue damage. Thus, protecting mitochondria is another method to reduce oxidative stress and then ameliorate inflammation caused by cell damage. NBP is proven to preserve normal cellular and mitochondrial function after OGD via stabilbing mitochondrial membrane potential (MMP), maintaining mitochondrial morphology, and boosting the activity of mitochondrial oxidative phosphorylation (OXPHOS) complexes (including complexes I-IV) and ATPase. NBP fixes the imbalance of protein that regulates mitochondrial fusion and division [77].

What is more, complex I, also named NADH-ubiquinone oxidoreductases, is linked with oxidative stress in mitochondria. Mutation leading to dysfunction of complex I has a positive effect on ROS production [130]. At the molecular level, NBP regulates the function of complex I to affect mitochondria and serve as an antioxidant agent mainly by competing for the sites (the 1,4-dihydropyridine) [131]. (S)-ZJM-289, as a novel NO-releasing derivative of NBP, attenuated OGD/R-induced mitochondrial dysfunction with the noticeable restoration of mitochondrial complex I/IV activity. It also markedly decreased ATP level, ROS generation, and [Ca2+]i accumulation in cortical neurons [132].

It has been reported that impaired mitochondrial biogenesis was alleviated by preserving mtDNA copy numbers [133]. TFAM has also been shown to maintain mtDNA and modulate the copy number [134]. NRF-1 is another crucial molecule in regulating energy supply and controlling mitochondrial biogenesis [135]. Tian et al. have found that NBP also significantly increased the contents of mitochondrial DNA (mtDNA) and mitochondrial biogenesis factors (NRF-1 and TFAM) after exposing cells to H2O2 [136], which further ascertains the function of NBP on promoting mitochondrial biogenesis. Therefore, NBP plays a role in stabilizing immunity and reducing inflammation by protecting the structure and function of mitochondria.

Given the above, besides the possible direct effects on immunity, NBP’s roles include sustentation of tissue function and prevention of uncontrolled immune responses, which contribute to, to some extent, inflammation and immune modulation.

3. Therapeutic Potential of NBP in Inflammatory and Immune-Mediated Diseases

3.1. NBP in Idiopathic Inflammatory Myopathies. Idiopathic inflammatory myopathies (IIM) are a group of autoimmune diseases characterized by muscle injury and other organ systems’ damage such as skin, lungs, and joints. Regardless of the different subtypes, the essential pathology of IIM is
skeletal muscle infiltration by T cells, B cells, and macrophages [137]. Some emerging provided a basis for considering NBP as a novel agent for the IIM treatment. Compared with the control group, clinical manifestations and inflammatory cell infiltration of experimental autoimmune myositis (EAM, a common animal model mimicking IIM in humans) were dose-dependently ameliorated in guinea pigs treated with NBP, which is through improving the Ca2+ ATPase activity of the muscle’s mitochondrial membrane and muscle’s plasma membrane. Regarding the NBP effects on T cell-associated cytokines, NBP remarkably reduced the expression of IFN-γ mRNA in muscle tissues and significantly elevated Foxp3 and RORγt mRNA expression levels [13]. Additionally, NBP exerted a protective effect by improving the antioxidant enzyme activity, reducing oxidative damage, and decreasing the apoptotic muscle cells in an EAM model [14]. Therefore, more research is needed in the future to explore the therapeutic effect of NBP in IIM and its underlying mechanism.

3.2. NBP in Multiple Sclerosis. Multiple sclerosis (MS) is an autoimmune-mediated neurodegenerative disease characterized by inflammatory demyelination with axonal transection. Elevated expression of PGAM5 (the components of necroptosis complex [138]) and worse inflammation induced by experimental autoimmune encephalomyelitis (EAE, a common-used animal model of MS) were reversed by NBP administration. Moreover, reexpression of PGAM5 counteracted the protective effect of NBP on the pathogenesis of EAE, in accordance with the results seen in vitro. It is implicated that NBP suppresses microglial cell growth, necroptosis, and inflammatory factor release by regulating PGAM5 [15].

3.3. Future Perspectives. NF-κB, as a critical role in the orchestration of the multifaceted inflammatory response, is active and exerts an effect in the production of inflammatory molecules in many inflammatory diseases, such as rheumatoid arthritis (RA), asthma, atherosclerosis, inflammatory bowel disease (IBD), or MS [22, 23]. It has been extensively studied that NF-κB is a target in treating inflammatory diseases. For example, artemisinin and its derivatives inhibit NF-κB by silencing these upstream pathways and/or directly binding to NF-κB, which alleviates the severity of systemic lupus erythematosus (SLE), autoimmune encephalitis (AE), dermatitis, IBD, autoimmune hepatitis, and autoimmune thyroiditis [139]. NBP has been demonstrated to downregulate NF-κB, implying NBP may be a potent and effective drug for the same autoimmune-mediated conditions via NF-κB pathways.

P38 signal is a central hub in arthritis and inflammation of the liver, kidney, brain, and lung, and it acts as a critical player in inflammatory diseases mediated by immune cells such as macrophages [40, 42, 52]. Accumulating evidence under human clinical trials shows that p38 inhibitors are a promising therapeutic strategy to control inflammatory diseases, for example, RA and chronic obstructive pulmonary disease (COPD) [52]. Thus, NBP with the function of regulating p38 activity probably has the potential to treat RA, COPD, and other immune-mediated diseases.
Due to the significance of HIF-1α, AMPK/SIRT1, PI3K/Ak, and Nrf-2/HO-1 in the inflammatory response and immune cell responses, pharmacologically targeting these signal pathways has been considered a treatment of many different immune-mediated diseases, including sepsis, IBD, RA, cancer, and autoimmune encephalomyelitis [140–143]. Similarly, NBP might be an ideal approach for sepsis, IBD, RA, cancer, and autoimmune encephalomyelitis as its ability to target HIF-1α, AMPK/SIRT1, PI3K/Ak, and Nrf-2/HO-1.

4. Conclusion

Although NBP is considered a compound with proven efficacy in treating ischemic stroke and a growing body of research concerning NBP’s effect on other diseases, there is a tremendous challenge of viable and effective transition from experimental to clinical practice. Besides, NBP’s potential mechanisms on modulating immunity and inflammation for immune- and inflammation-mediated disease remain unexplored. Based on these studies and data, we come to a novel perspective that NBP exerts anti-inflammation and immune regulation effects, at least partially, by modulating the signaling pathway discussed above (Figure 1) and alleviating oxidative stress. It potentially paves the way for a new strategy for immune-mediated diseases and inflammatory diseases to control immune responses. However, the dynamic changes of immune cells during the administration of NBP must be studied in much greater detail in the coming years. Thereby, further study will be needed to understand the precise molecular mechanisms of NBP concerning inflammation and immune response in the future.

The ways subsequently regulating immune cells are the following: (i) upregulating proinflammatory factors (TNF-α, IL-1β, IL-6, etc.); promoting macrophages/microglia to express a proinflammatory phenotype (M1) and prohibiting the expression of the anti-inflammatory phenotype (M2); regulating DC development, survival, and cytokine production; modulating B lymphocyte survival during their differentiation and in their activation; prohibiting induction of Treg and Th2; (ii) increasing proinflammatory mediators (TNF-α, PGE2, IL-1β, IL-1, IL-2, IL-3, IL-6, IL-8, IL-12, COX-2, etc.); regulating macrophage/microglia polarization; increasing apoptosis of immune cells; (iii) shifting macrophages/microglia toward M1 phenotype; modulating DC mature and immigration; regulating neutrophil extracellular trap formation and survival; differentiating and activating various T cells; reducing apoptosis of immune cells; (iv) downregulating proinflammatory molecules (COX-2, IL-1, etc.); accommodating T cell differentiation towards the anti-inflammatory phenotype; determining macrophage/microglia polarization; decreasing the production of ROS/NO; reducing autophagy; (v) reducing apoptosis and prolonging immune cell survival time; promoting phagocytosis and macrophage/microglia polarization via inducing related regulators (TGF-β, IL-10, and BMP-7); (vi) driving macrophage/microglia shift to M2 phenotype; inhibiting activation of Th and Tc; regulating the function of Treg.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] L.-y. Ao, Y.-Y. Yan, L. Zhou et al., “Immune cells after ischemic stroke onset: roles, migration, and target intervention,” Journal of Molecular Neuroscience, vol. 66, no. 3, pp. 342–355, 2018.

[2] X. Diao, P. Deng, C. Xie et al., “Metabolism and pharmacokinetics of 3-n-butylphthalide (NBP) in humans: the role of cytochrome P450s and alcohol dehydrogenase in biotransformation,” Drug Metabolism and Disposition, vol. 41, no. 2, pp. 430–444, 2013.

[3] H. Zhao, W. Yun, Q. Zhang et al., “Mobilization of circulating endothelial progenitor cells by dl-3-n-butylphthalide in acute ischemic stroke patients,” Journal of Stroke and Cerebrovascular Diseases, vol. 25, no. 4, pp. 752–760, 2016.

[4] H. L. Xu and Y. P. Feng, “Inhibitory effects of chiral 3-n-butylphthalide on inflammation following focal ischemic brain injury in rats,” Acta Pharmacologica Sinica, vol. 21, no. 5, pp. 433–438, 2000.

[5] R. Y. Yan, S. J. Wang, G. T. Yao, Z. G. Liu, and N. Xiao, “Analysis on the relationship and mechanism of high blood pressure and vascular aging on the condition that the gender and age matches,” European Review for Medical and Pharmacological Sciences, vol. 21, 3 Suppl, pp. 84–87, 2017.

[6] W. He, W. Zhou, and Z. Hu, “Chinese herbal extract dl-3n-butylphthalide: a commonly used drug for the treatment of ischemic stroke as a novel therapeutic approach to treat neurodegenerative diseases,” Neural Regeneration Research, 2011.

[7] S.-J. Liao, J.-W. Lin, Z. Pei, C.-L. Liu, J.-S. Zeng, and R.-X. Huang, “Enhanced angiogenesis with _dl_ -3n-butylphthalide treatment after focal cerebral ischemia in RHRSP,” Brain Research, vol. 1289, pp. 69–78, 2009.

[8] J. Ye, L. Zhai, S. Zhang et al., “DL-3-n-Butylphthalide inhibits platelet activation via inhibition of cPLA2-mediated TXA2 synthesis and phosphodiesterase,” Platelets, vol. 26, no. 8, pp. 736–744, 2015.

[9] G. Dong and Y.-P. Feng, “Effects of NBP on ATPase and antioxidative enzymes activities and lipid peroxidation in transient focal cerebral ischemic rats,” Zhongguo Yi Xue Ke Xue Yuan Xue Bao, vol. 24, no. 1, pp. 93–97, 2002.

[10] J. Li, Y. Li, M. Ogle et al., “dl-3- _n_ -Butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway,” Brain Research, vol. 1359, pp. 216–226, 2010.

[11] X. Q. Chen, K. Qiu, H. Liu, Q. He, J. H. Bai, and W. Lu, “Application and prospects of butylphthalide for the...
treatment of neurologic diseases,” Chinese Medical Journal, vol. 132, no. 12, pp. 1467–1477, 2019.

[12] I. A. Abdoulaye and Y. J. Guo, “A review of recent advances in neuroprotective potential of 3-N-butylphthalide and its derivatives,” Biomed Research International, vol. 2016, 9 pages, 2016.

[13] J. Chen, J. Wang, J. Zhang, and C. Pu, “Effect of butylphthalide intervention on experimental autoimmune myositis in guinea pigs,” Experimental and Therapeutic Medicine, vol. 15, no. 1, pp. 152–158, 2018.

[14] J. Chen, J. Wang, J. Zhang, and C. Pu, “3-Butylphthalide reduces the oxidative damage of muscles in an experimental autoimmune myositis animal model,” Experimental and Therapeutic Medicine, vol. 14, no. 3, pp. 2085–2093, 2017.

[15] Y. Wang, Y. Bi, Z. Xia et al., “Butylphthalide ameliorates experimental autoimmune encephalomyelitis by suppressing PGAM5-induced necroptosis and inflammation in microglia,” Biochemical and Biophysical Research Communications, vol. 497, no. 1, pp. 80–86, 2018.

[16] L. Wang and X.-J. He, “Butylphthalide has an anti-inflammatory role in spinal cord injury by promoting macrophage/microglia M2 polarization via p38 phosphorylation,” Spine (Phila Pa 1976), vol. 45, no. 17, pp. E1066–E1076, 2020.

[17] F. Li, Q. Ma, H. Zhao et al., “L-3-n-Butylphthalide reduces ischemic stroke injury and increases M2 microglial polarization,” Metabolic Brain Disease, vol. 33, no. 6, pp. 1995–2003, 2018.

[18] M. Srivasan and D. K. Lahiri, “Significance of NF-κB as a pivotal therapeutic target in the neurodegenerative pathologies of Alzheimer’s disease and multiple sclerosis,” Expert Opinion on Therapeutic Targets, vol. 19, no. 4, pp. 471–487, 2015.

[19] Q. Zhang, M. J. Lenardo, and D. Baltimore, “30 years of NF-κB: a blossoming of relevance to human pathobiology,” Cell, vol. 168, no. 1-2, pp. 37–57, 2017.

[20] M. Karin, “NF-κB as a critical link between inflammation and cancer,” Cold Spring Harbor Perspectives in Biology, vol. 1, no. 5, article a001411, 2009.

[21] R. G. Baker, M. S. Hayden, and S. Ghosh, “NF-κB, inflammation, and metabolic disease,” Cell Metabolism, vol. 13, no. 1, pp. 11–22, 2011.

[22] W.-N. Lin, S.-F. Luo, C.-W. Lee, C.-C. Wang, J.-S. Wang, and M.-M. Yang, “Involvement of MAPKs and NF-κB in LPS-induced VCAM-1 expression in human tracheal smooth muscle cells,” Cell Signalling, vol. 19, no. 6, pp. 1258–1267, 2007.

[23] G. Yang and H. J. An, “β-Sitosteryl-3-O-β-glucopyranoside isolated from the bark of Sorbus commixta ameliorates pro-inflammatory mediators in RAW 264.7 macrophages,” Immunopharmacology and Immunotoxicology, vol. 36, no. 1, pp. 70–77, 2014.

[24] H. M. Wang, T. Zhang, J. K. Huang, and X. J. Sun, “3-N-butylphthalide (NBP) attenuates the amyloid-β-induced inflammatory responses in cultured astrocytes via the nuclear factor-κB signaling pathway,” Cellular Physiology and Biochemistry, vol. 32, no. 1, pp. 235–242, 2013.

[25] X. Feng, Y. Peng, M. Liu, and L. Cui, “DI-3-n-butylphthalide extends survival by attenuating glial activation in a mouse model of amyotrophic lateral sclerosis,” Neuropsychopharmacology, vol. 62, no. 2, pp. 1004–1010, 2012.

[26] M. Yang, R. Dang, P. Xu et al., “DI-3-n-Butylphthalide improves lipopolysaccharide-induced depressive-like behavior in rats: involvement of Nrf2 and NF-κB pathways,” Psychopharmacology, vol. 235, no. 9, pp. 2573–2585, 2018.

[27] Y. Zhao, J. H. Lee, D. Chen et al., “DL-3-n-Butylphthalide induced neuroprotection, regenerative repair, functional recovery and psychological benefits following traumatic brain injury in mice,” Neurochemistry International, vol. 111, pp. 82–92, 2017.

[28] M. Li, N. Meng, X. Guo et al., “DL-3-n-Butylphthalide promotes remyelination and suppresses inflammation by regulating AMPK/SIRT1 and STAT3/NF-κB signaling in chronic cerebral hypoperfusion,” Frontiers in Aging Neuroscience, vol. 12, 2020.

[29] Y. Wu, L. Dong, Q. Huang et al., “Multiple functional therapeutic effects of DL-3-n-butylphthalide in the cuprizone model of demyelination,” Life Sciences, vol. 232, article 116501, 2019.

[30] H.-J. Kim, H.-I. Joe, Z. Zhang et al., “Anti-inflammatory effect of _Acalypha australis_ L. via suppression of NF-κB signaling in LPS-stimulated RAW 264.7 macrophages and LPS-induced septic mice,” Molecular Immunology, vol. 119, pp. 123–131, 2020.

[31] P. Zhang, Z.-f. Guo, Y.-m. Xu, Y.-s. Li, and J.-g. Song, “N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/NF-κB signaling pathway,” Biomedicine & Pharmacotherapy, vol. 83, pp. 658–666, 2016.

[32] Z. He, Y. Zhou, L. Lin et al., “DL-3-n-Butylphthalide attenuates acute inflammatory activation in rats with spinal cord injury by inhibiting microglial TLR4/NF-κB signalling,” Journal of Cellular and Molecular Medicine, vol. 21, no. 11, pp. 3010–3022, 2017.

[33] U. Cho, B. Kim, S. Kim, Y. Han, and Y. S. Song, “Pro-inflammatory M1 macrophage enhances metastatic potential of ovarian cancer cells through NF-κB activation,” Molecular Carcinogenesis, vol. 57, no. 2, pp. 235–242, 2018.

[34] Y. Chen, T. Wu, H. Li et al., “DL-3-n-Butylphthalide exerts dopaminergic neuroprotection through inhibition of neuroinflammation,” Frontiers in Aging Neuroscience, vol. 11, 2019.

[35] F. Ouaz, J. Arron, Y. Zheng, Y. Choi, and A. A. Beg, “Dendritic cell development and survival require distinct NF-kappaB subunits,” Immunity, vol. 16, no. 2, pp. 257–270, 2002.

[36] M. Rescigno, M. Martino, C. L. Sutherland, M. R. Gold, and P. Ricciardi-Castagnoli, “Dendritic cell survival and maturation are regulated by different signaling pathways,” The Journal of Experimental Medicine, vol. 188, no. 11, pp. 2175–2180, 1998.

[37] E. Martin, B. O’Sullivan, P. Low, and R. Thomas, “Antigen-specific suppression of a primed immune response by dendritic cells mediated by regulatory T cells secreting interleukin-10,” Immunity, vol. 18, no. 1, pp. 155–167, 2003.

[38] S. Yoshimura, J. Bondeson, F. M. Brennan, B. M. J. Foxwell, and M. Feldmann, “Role of NFkappaB in antigen presentation and development of regulatory T cells elucidated by treatment of dendritic cells with the proteasome inhibitor PSI,” European Journal of Immunology, vol. 31, no. 6, pp. 1883–1893, 2001.

[39] S. Gerondakis and A. Strasser, “The role of Rel/NF-kappaB transcription factors in B lymphocyte survival,” Seminars in Immunology, vol. 15, no. 3, pp. 159–166, 2003.

[40] S. Kumar, J. Boehm, and J. C. Lee, “P 38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory
diseases,” Nature Reviews. Drug Discovery, vol. 2, no. 9, pp. 717–726, 2003.

[41] A. Bohush, G. Niewiadomska, and A. Filippek, “Role of mitogen-activated protein kinase signaling in Parkinson’s disease,” International Journal of Molecular Sciences, vol. 19, no. 10, p. 2973, 2018.

[42] A. Mavropoulos, T. Orfanidou, C. Liaskos et al., “p38 mitogen-activated protein kinase (p38 MAPK)-mediated autophagy: lessons to learn from ANCA vasculitis and pempigus vulgaris,” Autoimmunity Reviews, vol. 12, no. 5, pp. 580–590, 2013.

[43] G. Kheiri, M. Dolatshahi, F. Rahmani, and N. Rezaei, “Role of p38/MAPKs in Alzheimer’s disease: implications for amyloid beta toxicity targeted therapy,” Reviews in the Neurosciences, vol. 30, pp. 9–30, 2018.

[44] R. Liu and J. D. Molkentin, “Regulation of cardiac hypertrophy and remodeling through the dual-specificity MAPK phosphatases (DUSPs),” Journal of Molecular and Cellular Cardiology, vol. 101, pp. 44–49, 2016.

[45] K. C. Nandipati, S. Subramanian, and D. K. Agrawal, “Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance,” Molecular and Cellular Biochemistry, vol. 426, no. 1-2, pp. 27–45, 2017.

[46] M. S. Gee, S. W. Kim, N. Kim et al., “A novel and selective p38 mitogen-activated protein kinase inhibitor attenuates LPS-induced neuroinflammation in BV2 microglia and a mouse model,” Neurochemical Research, vol. 43, no. 12, pp. 2362–2371, 2018.

[47] L.-L. Yang, Y. Zhou, W.-D. Tian et al., “Electromagnetic pulse activated brain microglia via the p38 MAPK pathway,” Neurotoxicology, vol. 52, pp. 144–149, 2016.

[48] J.-E. Kim, H. Park, S.-H. Choi, M.-J. Kong, and T.-C. Kang, “Roscovitine attenuates microglia activation and monocyte infiltration via p38 MAPK inhibition in the rat frontoparietal cortex following status epilepticus,” Cell, vol. 8, no. 7, p. 746, 2019.

[49] J. R. Perea, J. Ávila, and M. Bologs, “Dephosphorylated rather than hyperphosphorylated tau triggers a pro-inflammatory profile in microglia through the p38 MAPK pathway,” Experimental Neurology, vol. 310, pp. 14–21, 2018.

[50] Y. Yang, R. Zhang, F. Xia et al., “LPS converts Gl-1’CD115’ myeloid-derived suppressor cells from M2 to M1 via p38 MAPK,” Experimental Cell Research, vol. 319, pp. 1774–1783, 2013.

[51] A. Risco, C. del Fresno, A. Mambol et al., “p38γ and p38δ kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation,” Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 28, pp. 11200–11205, 2012.

[52] Y. Yang, S. C. Kim, T. Yu et al., “Functional roles of p38 mitogen-activated protein kinase in macrophage-mediated inflammatory responses,” Mediators of Inflammation, vol. 2014, 13 pages, 2014.

[53] W. S. Yang, Y. C. Park, J. H. Kim et al., “Nanostructured, self-assembling peptide K5 blocks TNF-α and PGE2Production by suppression of the AP-1/p38 pathway,” Mediators of Inflammation, vol. 2012, 8 pages, 2012.

[54] S. E. Byeon, J. Lee, B. C. Yoo et al., “p38-Targeted inhibition of interleukin-12 expression by ethanol extract from Cordyceps bassianain lipopolysaccharide-activated macrophages,” Immunopharmacology and Immunotoxicology, vol. 33, no. 1, pp. 90–96, 2011.

[55] J. Garcia, B. Lemercier, S. Roman-Roman, and G. Rawadi, “Two-stage activation for alpha5beta1 integrin binding to surface-adsorbed fibronectin,” The Journal of Biological Chemistry, vol. 273, no. 52, pp. 34710–34715, 1998.

[56] A. Amirouche, H. Tadesse, J. A. Lunde, G. Bélanger, J. Côté, and B. J. Jasmin, “Activation of p38 signaling increases utrophin A expression in skeletal muscle via the RNA-binding protein KSRP and inhibition of AU-rich element-mediated mRNA decay: implications for novel DMD therapeutics,” Human Molecular Genetics, vol. 22, no. 15, pp. 3093–3111, 2013.

[57] N. Xie, H. Li, D. Wei et al., “Glycogen synthase kinase-3 and p38 MAPK are required for opioid-induced microglia apoptosis,” Neuropharmacology, vol. 59, no. 6, pp. 444–451, 2010.

[58] G. L. Semenza, “HIF-1 and tumor progression: pathophysiology and therapeutics,” Trends in Molecular Medicine, vol. 8, no. 4, pp. S62–S67, 2002.

[59] J. Min, X. Huo, L. Xiang, Y. Qin, K. Chai, and B. Wu, “Protective effect of dl-3-n-butylphthalide on learning and memory impairment induced by chronic intermittent hypoxia-hypercapnia exposure,” Scientific Reports, vol. 4, 2014.

[60] W. Yang, L. Li, R. Huang, Z. Pei, S. Liao, and J. Zeng, “Hypoxia-inducible factor-1alpha mediates protection of DL-3-n-butylphthalide in brain microvascular endothelial cells against oxygen glucose deprivation-induced injury,” Neural Regeneration Research, vol. 7, no. 12, pp. 948–954, 2012.

[61] L. Li, B. Zhang, Y. Tao et al., “dl-3-N-Butylphthalide protects endothelial cells against oxidative/nitrosative stress, mitochondrial damage and subsequent cell death after oxygen glucose deprivation in vitro,” Brain Research, vol. 1290, pp. 91–101, 2009.

[62] T. Cramer, Y. Yamanishii, B. E. Clausen et al., “HIF-1alpha is essential for myeloid cell-mediated inflammation,” Cell, vol. 112, no. 5, pp. 645–657, 2003.

[63] N. Takeda, E. L. O’Dea, A. Doedens, J. W. Kim, A. Weidemann, and C. Stockmann, “Differential activation and antagonistic function of HIF-1alpha isoforms in macrophages are essential for NO homeostasis,” Genes & Development, vol. 24, no. 5, pp. 491–501, 2010.

[64] T. Wang, H. Liu, G. Lian, S.-Y. Zhang, X. Wang, and C. Jiang, “HIF1α-induced glycolysis metabolism is essential to the activation of inflammatory macrophages,” Mediators of Inflammation, vol. 2017, 10 pages, 2017.

[65] C. L. Lyons and H. M. Roche, “Nutritional modulation of AMPK-impact upon metabolic-inflammation,” International Journal of Molecular Sciences, vol. 19, no. 10, pp. 238–242, 2018.

[66] E. V. Dang, J. Barbi, H. Y. Yang et al., “Control of TH17/Treg balance by hypoxia-inducible factor 1,” Cell, vol. 146, no. 5, pp. 772–784, 2011.

[67] J. Liu, X. Zhang, K. Chen et al., “CCR7 chemokine receptor-inducible Inc-Dp3 restrains dendritic cell migration by inhibiting HIF-1α-mediated glycolysis,” Immunity, vol. 50, no. 3, pp. 600–615.e15, 2019.

[68] T. Köhler, B. Reizis, R. S. Johnson, H. Weighardt, and I. Förster, “Influence of hypoxia-inducible factor 1α on dendritic cell differentiation and migration,” European Journal of Immunology, vol. 42, no. 5, pp. 1226–1236, 2012.
[69] S. R. Walmsley, C. Print, N. Farahi et al., "Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity," The Journal of Experimental Medicine, vol. 201, no. 1, pp. 105–115, 2005.

[70] K. I. Mecklenburgh, S. R. Walmsley, A. S. Cowburn et al., "Involvement of a ferroprotein sensor in hypoxia-mediated inhibition of neutrophil apoptosis," Blood, vol. 100, no. 8, pp. 3008–3016, 2002.

[71] E. P. Cummins, C. E. Keogh, D. Crean, and C. T. Taylor, "The role of HIF in immunity and inflammation," Molecular Aspects of Medicine, vol. 47–48, pp. 24–34, 2016.

[72] G. J. Baay-Guzman, M. A. Duran-Padilla, J. Rangel-Santiago et al., "Dual role of hypoxia-inducible factor 1 alpha in experimental pulmonary tuberculosis: its implication as a new therapeutic target," Future Microbiology, vol. 13, pp. 785–798, 2018.

[73] S. T. Schäfer, S. Frede, S. Winning et al., "Hypoxia-inducible factor and target gene expression are decreased in patients with sepsis: prospective observational clinical and cellular studies," Anesthesiology, vol. 118, no. 6, pp. 1426–1436, 2013.

[74] B. Dasgupta and J. Milbrandt, "AMP-activated protein kinase phosphorylates retinoblastoma protein to control mammalian brain development," Developmental Cell, vol. 16, no. 2, pp. 256–260, 2009.

[75] S. I. Imai, C. M. Armstrong, M. Kaeberlein, and L. Guarente, "Transcriptional silencing and longevity protein Sir 2 is an NAD-dependent histone deacetylase," Nature, vol. 403, no. 6771, pp. 795–800, 2000.

[76] H. C. Chang and L. Guarante, "SIRT1 and other sirtuins in metabolism," Trends in Endocrinology and Metabolism, vol. 25, no. 3, pp. 138–145, 2014.

[77] N. Chen, Z. Zhou, J. Li et al., "3-butyphthalide exerts neuroprotective effects by enhancing anti-oxidation and attenuating mitochondrial dysfunction in an in vitro model of ischemic stroke," Drug Design, Development and Therapy, vol. 12, pp. 4261–4271, 2018.

[78] J. Rolf, M. Zarrouk, D. K. Finlay, M. Foretz, B. Viollet, and D. A. Cantrell, "AMPKα1: a glucose sensor that controls CD8 T-cell memory," European Journal of Immunology, vol. 43, no. 4, pp. 889–896, 2013.

[79] J. Blagh, F. Coulombe, E. E. Vincent et al., "The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo," Immunity, vol. 42, no. 1, pp. 41–54, 2015.

[80] R. D. Michalek, V. A. Gerriets, S. R. Jacobs, A. N. Macintyre, N. J. Mac Iver, and E. F. Mason, "Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4 + T cell subsets," Journal of Immunology, vol. 186, no. 6, pp. 3299–3303, 2011.

[81] S. M. Poznanski, N. G. Barra, A. A. Ashkar, and J. D. Schertzer, "Immunometabolism of T cells and NK cells: metabolic control of effector and regulatory function," Inflammation Research, vol. 67, no. 10, pp. 813–828, 2018.

[82] J. F. Brifna, K. Anevska, L. Chen, M. E. Wlodek, and T. Romano, "Metformin administration in pregnant high-fat fed rats improves metabolic function and adiposity," Obesity Research & Clinical Practice, vol. 13, no. 3, p. 279, 2019.

[83] J. Wang, Z. Li, L. Gao, Y. Qi, H. Zhu, and X. Qin, "The regulation effect of AMPK in immune related diseases," Science China. Life Sciences, vol. 61, no. 5, pp. 523–533, 2018.

[84] G. Pilon, P. Dallaire, and A. Marette, "Inhibition of inducible nitric-oxide synthase by activators of AMP-activated protein kinase: a new mechanism of action of insulin-sensitizing drugs," The Journal of Biological Chemistry, vol. 279, no. 20, pp. 20767–20774, 2004.

[85] H. Wen, D. Gris, Y. Lei et al., "Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling," Nature Immunology, vol. 12, no. 5, pp. 408–415, 2011.

[86] J. Zhao, J. Liu, E. Xu, Y. Liu, A. Xie, and H. Xiong, "dl-3-n-Butylphthalide attenuation of methamphetamine-induced neurotoxicity in SH-SY5Y neuroblastoma cells," Life Sciences, vol. 165, pp. 16–20, 2016.

[87] Z.-Y. Ye, H.-Y. Xing, B. Wang, M. Liu, and P.-Y. Lv, "DL-3-n-Butylphthalide protects the blood-brain barrier against ischemia/hypoxia injury via upregulation of tight junction proteins," Chinese Medical Journal, vol. 132, no. 11, pp. 1344–1353, 2019.

[88] D.-P. Chen, S.-H. Hou, Y.-G. Chen, M.-S. Chen, Z.-Z. Hu, and Z.-J. Zhang, "L-Butyl phthalalim improves neuronal function of vascular dementia mice by regulating the PI3K/AKT signaling pathway," European Review for Medical and Pharmacological Sciences, vol. 22, 2018.

[89] Q. Yu, L. Dong, Y. Li, and G. Liu, "SIRT1 and HIF1α signaling in metabolism and immune responses," Cancer Letters, vol. 418, pp. 20–26, 2018.

[90] X. Chen, Y. Lu, Z. Zhang, J. Wang, H. Yang, and G. Liu, "Intercellular interplay between Sirt1 signalling and cell metabolism in immune cell biology," Immunology, vol. 145, no. 4, pp. 455–467, 2015.

[91] A. Belacosa, S. S. Testa, and P. Tsichlis, "A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region," Science, vol. 254, no. 5029, pp. 274–277, 1991.

[92] T. F. Franke, Y. S. Il, T. O. Chan, K. Datta, A. Kazlauskas, and D. K. Morrison, "The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase," Cell, vol. 81, no. 5, pp. 727–736, 1995.

[93] W. Li, D. Wei, J. Lin et al., "DL-3-n-Butylphthalide reduces cognitive impairment induced by chronic cerebral hypoperfusion through GDNF/GFRα1/ret signaling preventing hippocampal neuron apoptosis," Frontiers in Cellular Neuroscience, vol. 13, 2019.

[94] W. Wang, T. Wang, S. Bai, Z. Chen, X. Qi, and P. Xie, "DL-3-N-Butylphthalide attenuates mouse behavioral deficits of chronic social defeat stress by regulating energy metabolism via AKT/CREB signaling pathway," Translational Psychiatry, vol. 10, no. 1, p. 49, 2020.

[95] B. Sun, M. Feng, X. Tian et al., "dl-3-n-Butylphthalide protects rat bone marrow stem cells against hydrogen peroxide-induced cell death through antioxidation and activation of PI3K-Akt pathway," Neuroscience Letters, vol. 516, no. 2, pp. 247–252, 2012.

[96] X.-L. Lu, D. Luo, X.-L. Yao et al., "dl-3n-Butylphthalide promotes angiogenesis via the extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase/Akt-endothelial nitric oxide synthase signaling pathways," Journal of Cardiovascular Pharmacology, vol. 59, no. 4, pp. 352–362, 2012.

[97] J. Xu, Y. Huai, N. Meng et al., "l-3-N-Butylphthalide activates Akt/mTOR signaling, inhibits neuronal apoptosis and autophagy and improves cognitive impairment in mice with repeated cerebral ischemia–reperfusion injury," Neurochemical Research, vol. 42, no. 10, pp. 2968–2981, 2017.
[98] J. P. Luyendyk, G. A. Schabacker, M. Tencati, T. Holscher, R. Pawlinski, and N. Mackman, "Genetic analysis of the role of the PI3K-Akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages," *Journal of Immunology*, vol. 180, no. 6, pp. 4218–4226, 2008.

[99] M. López-Peláez, I. Soria-Castro, L. Boscà, M. Fernández, and S. Alemany, "Cot/tp2 activity is required for TLR-induced activation of the Akt p70S6K pathway in macrophages: implications for NO synthase 2 expression," *European Journal of Immunology*, vol. 41, no. 6, pp. 1733–1741, 2011.

[100] D. Gong, W. Shi, S. Yi, H. Chen, J. Groffen, and N. Heisterkamp, "TGFβ signaling plays a critical role in promoting alternative macrophage activation," *BMJ Immunology*, vol. 13, p. 31, 2012.

[101] H. J. Park, S. J. Lee, S. H. Kim et al., "IL-10 inhibits the starvation induced autophagy in macrophages via class I phosphatidylinositol 3-kinase (PI3K) pathway," *Molecular Immunology*, vol. 48, no. 4, pp. 720–727, 2011.

[102] C. Rocher and D. K. Singla, "SMAD-PI3K-Akt-mTOR pathway mediates BMP-7 polarization of monocytes into M2 macrophages," *PLoS One*, vol. 8, no. 12, 2013.

[103] H. Shiratsuchi and M. D. Basson, "Akt 2, but not Akt 1 or Akt 3 mediates pressure-stimulated serum-opsonized latex bead phagocytosis through activating mTOR and p70 S6 kinase," *Journal of Cellular Biochemistry*, vol. 102, no. 2, pp. 353–367, 2007.

[104] M. J. Rane, P. Y. Coxon, D. W. Powell et al., "p38 kinase-dependent MAPKAP-K2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils," *The Journal of Biological Chemistry*, vol. 276, no. 5, pp. 3517–3523, 2001.

[105] Y. Xu, F. Loison, and H. R. Luo, "Neutrophil spontaneous death is mediated by down-regulation of autocrine signaling through GPCR, PI3K gamma, ROS, and actin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 7, pp. 2950–2955, 2010.

[106] S. J. Gardai, D. A. Hildeman, S. K. Frankel et al., "xorPhosphorylation of Bax Ser185 by Akt regulates its activity and apoptosis in neutrophils," *The Journal of Biological Chemistry*, vol. 279, no. 20, pp. 21085–21095, 2004.

[107] R. A. Linker, D.-H. Lee, S. Ryan et al., "Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway," *Brain*, vol. 134, Part 3, pp. 678–692, 2011.

[108] K.-A. Jung and M.-K. Kwak, "The Nrf2 system as a potential target for the development of indirect antioxidants," *Molecules*, vol. 15, no. 10, pp. 7266–7291, 2010.

[109] R. Tenhunen, H. S. Marver, and R. Schmid, "Microsomal heme oxygenase. Characterization of the enzyme," *The Journal of Biological Chemistry*, vol. 244, no. 23, pp. 6388–6394, 1969.

[110] Y.-J. Zhao, Y. Nai, Q.-S. Ma, D.-J. Song, Y.-B. Ma, and L.-H. Zhang, "DL-3-n-Butylphthalide protects the blood brain barrier of cerebral infarction by activating the Nrf-2/HO-1 signaling pathway in mice," *European Review for Medical and Pharmacological Sciences*, vol. 22, 2018.

[111] H. Qu, H. Wu, J. Ma et al., "DL-3-n-Butylphthalide reduces atrial fibrillation susceptibility by inhibiting atrial structural remodeling in rats with heart failure," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 391, no. 3, pp. 323–334, 2018.

[112] A. Paine, B. Eiz-Vesper, R. Blascozy, and S. Immenschuh, "Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential," *Biochemical Pharmacology*, vol. 80, no. 12, pp. 1895–1903, 2010.

[113] P. Mandal, B. T. Pratt, M. Barnes, M. R. McMullen, and L. E. Nagy, "Molecular mechanism for adiponectin-dependent M2 macrophage polarization," *The Journal of Biological Chemistry*, vol. 286, no. 15, pp. 13460–13469, 2011.

[114] Y. Naito, T. Takagi, and Y. Higashimura, "Heme oxygenase-1 and anti-inflammatory M2 macrophages," *Archives of Biochemistry and Biophysics*, vol. 564, pp. 83–88, 2014.

[115] H.-O. Pae, G.-S. Oh, B.-M. Choi et al., "Carbon monoxide produced by heme oxygenase-1 suppresses T cell proliferation via inhibition of IL-2 production," *Journal of Immunology*, vol. 172, no. 8, pp. 4744–4751, 2004.

[116] B. M. Choi, H. O. Pae, Y. R. Jeong, Y. M. Kim, and H. T. Chung, "Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression," *PLoS One*, vol. 8, no. 3, pp. 1066–1071, 2015.

[117] Y. Liu, P. Li, J. Lu et al., "Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis," *Journal of Immunology*, vol. 181, no. 3, pp. 1887–1897, 2008.

[118] R. Song, Z. Zhou, P. K. M. Kim et al., "Carbon monoxide promotes Fas/CD95-induced apoptosis in Jurkat cells," *The Journal of Biological Chemistry*, vol. 279, no. 43, pp. 44327–44334, 2004.

[119] H. Kono and K. L. Rock, "How dying cells alert the immune system to danger," *Nature Reviews. Immunology*, vol. 8, no. 4, pp. 279–289, 2008.

[120] E. Ambrozewicz, P. Wójcik, A. Wroński et al., "Pathophysiological alterations of redox signaling and endocannabinoid system in granulocytes and plasma of psoriatic patients," *Cell*, vol. 7, no. 10, p. 159, 2018.

[121] P. Wójcik, M. Biernacki, A. Wroński et al., "Altered lipid metabolism in blood mononuclear cells of psoriatic patients indicates differential changes in psoriasis vulgaris and psoriatic arthritis," *International Journal of Molecular Sciences*, vol. 20, no. 17, p. 4249, 2019.

[122] M. Jaganjac, A. Cipak, R. J. Schaur, and N. Žarkovíc, "Pathophysiology of neutrophil-mediated extracellular redox reactions," *Frontiers in Bioscience*, vol. 21, no. 4, pp. 839–855, 2016.

[123] P. Wójcik, N. Žarkovíc, A. Gegotek, and E. Skrzydlewska, "Involvement of metabolic lipid mediators in the regulation of apoptosis," *Biomolecules*, vol. 10, no. 3, p. 402, 2020.

[124] S. I. Ruzví and P. K. Mauyra, "Alterations in antioxidant enzymes during aging in humans," *Molecular Biotechnology*, vol. 37, no. 1, pp. 58–61, 2007.

[125] Z. Tian, J. Wang, Y. Wang, M. Zhang, and Y. Zhou, "Effects of butylphthalide on cognitive decline in diabetic rats," *Molecular Medicine Reports*, vol. 16, no. 6, pp. 9131–9136, 2017.

[126] J. M. Lee, M. J. Calkins, K. Chan, Y. W. Kan, and J. A. Johnson, "Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis," *The Journal of Biological Chemistry*, vol. 278, no. 14, pp. 12029–12038, 2003.

[127] S. Chrissobolis, A. A. Miller, G. R. Drummond, B. K. Kemp-Harper, and C. G. Sobey, "Oxidative stress and endothelial
dysfunction in cerebrovascular disease,” Frontiers in Biosciences, vol. 16, no. 1, pp. 1733–1745, 2011.

[128] T. Eisenberg, S. Büttner, G. Kroemer, and F. Madeo, “The mitochondrial pathway in yeast apoptosis,” Apoptosis, vol. 12, no. 5, pp. 1011–1023, 2007.

[129] I. M. Cojocaru, M. Cojocaru, V. Sapira, and A. Ionescu, “Evaluation of oxidative stress in patients with acute ischemic stroke,” Romanian Journal of Internal Medicine, vol. 51, no. 2, pp. 97–106, 2013.

[130] Y. Liu, G. Fiskum, and D. Schubert, “Generation of reactive oxygen species by the mitochondrial electron transport chain,” Journal of Neurochemistry, vol. 80, no. 5, pp. 780–787, 2002.

[131] Y. Wang, W. Qi, L. Zhang et al., “The novel targets of DL-3-n-butylphthalide predicted by similarity ensemble approach in combination with molecular docking study,” Quantitative Imaging in Medicine and Surgery, vol. 7, no. 5, pp. 532–536, 2017.

[132] Q. Zhao, C. Zhang, X. Wang, L. Chen, H. Ji, and Y. Zhang, “(_S_)-ZJM-289, a nitric oxide-releasing derivative of 3-n-butylphthalide, protects against ischemic neuronal injury by attenuating mitochondrial dysfunction and associated cell death,” Neurochemistry International, vol. 60, no. 2, pp. 134–144, 2012.

[133] T. Inoue, M. Ikeda, T. Ide et al., “Twinkle overexpression prevents cardiac rupture after myocardial infarction by alleviating impaired mitochondrial biogenesis,” American Journal of Physiology-Heart and Circulatory Physiology, vol. 311, no. 3, pp. H509–H519, 2016.

[134] D. Kang and N. Hamasaki, “Mitochondrial transcription factor A in the maintenance of mitochondrial DNA,” Annals of the New York Academy of Sciences, vol. 1042, no. 1, pp. 101–108, 2005.

[135] C. J. McLeod, A. P. Jeyabalan, J. O. Minners, R. Cleverger, R. F. Hoyt, and M. N. Sack, “Delayed ischemic preconditioning activates nuclear-encoded electron-transfer-chain gene expression in parallel with enhanced postanoxic mitochondrial respiratory recovery,” Circulation, vol. 110, no. 5, pp. 534–539, 2004.

[136] X. Tian, W. He, R. Yang, and Y. Liu, “DL-3-n-Butylphthalide protects the heart against ischemic injury and H9c2 cardiomyoblasts against oxidative stress: involvement of mitochondrial function and biogenesis,” Journal of Biomedical Science, vol. 24, no. 1, p. 38, 2017.

[137] L. G. Rider and F. W. Miller, “Deciphering the clinical presentations, pathogenesis, and treatment of the idiopathic inflammatory myopathies,” Journal of the American Medical Association, vol. 305, no. 2, pp. 183–190, 2011.

[138] W. Lu, J. Sun, J. S. Yoon et al., “Mitochondrial protein PGAM5 regulates mitophagic protection against cell necroptosis,” PLoS One, vol. 11, no. 1, article e0147792, 2016.

[139] T. Efferth and F. Oesch, “The immunosuppressive activity of artemisinin-type drugs towards inflammatory and autoimmune diseases,” Medicinal Research Reviews, vol. 41, no. 6, pp. 3023–3061, 2021.

[140] A. F. McGGettrick and L. A. J. O’Neill, “The role of HIF in immunity and inflammation,” Cell Metabolism, vol. 32, pp. 524–536, 2020.

[141] J. Wang, C. Zhao, P. Kong et al., “Methylene blue alleviates experimental autoimmune encephalomyelitis by modulating AMPK/SIRT1 signaling pathway and Th17/Treg immune response,” Journal of Neuroimmunology, vol. 299, pp. 45–52, 2016.