Zhang and Yang: TLR4 and NLRP3 roles in hyperuricaemia nephropathy

Targeting Toll-like receptor 4 (TLR4) and the NLRP3 inflammasome: Novel and emerging therapeutic targets for hyperuricaemia nephropathy

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ABSTRACT

The clinical manifestation of hyperuricaemia, known as hyperuricaemia nephropathy, is relatively common. Its pathophysiology is largely based on chronic inflammation in circulatory and renal tissues. Toll-like receptor 4 (TLR4), a subclass of innate immune receptors, detects both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), initiating inflammatory and immune responses that lead to the release of pro-inflammatory cytokines interleukin (IL) 1β and tumor necrosis factor alpha (TNF-α). These cytokines are pivotal in renal inflammation, especially in conditions like hyperuricaemia, acute renal injury, ischemia-reperfusion injury, and acute renal failure. The nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain-containing 3 (NLRP3) inflammasome, an essential component of the innate immune signaling complex, plays a central role in inflammation. It finely regulates the activation of caspase-1 and the production and secretion of the pro-inflammatory cytokine IL-1β, mediating and amplifying the inflammatory cascade response. Activation of TLR4 indirectly promotes the assembly of the NLRP3 inflammasome by regulating the nuclear factor kappa B (NF-κB) signaling pathway, thereby amplifying the inflammatory process and playing a significant pro-inflammatory role in hyperuricaemia nephropathy. TLR4 and NLRP3 inflammasome are anticipated to be novel markers and therapeutic targets for assessing treatment efficacy and prognosis in hyperuricaemia nephropathy. This paper provides a comprehensive overview of the structural composition and biological functions of TLR4 and NLRP3 inflammasome and systematically reviews their relevance in the
pathogenesis of hyperuricaemia nephropathy.

**Keywords:** Hyperuricaemia nephropathy; pathogenesis; inflammation; Toll-like receptor 4 (TLR4); nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain-containing 3 (NLRP3).
INTRODUCTION

Uric acid (UA) is the end product of purine metabolism. Abnormal serum UA (sUA) levels may occur due to changes in uric acid production or excretion. Physiological concentrations of UA have anti-inflammatory and chondroprotective effects [1]. However, excessive accumulation results in hyperuricaemia and the deposition of urate crystals in various tissues, including joints and kidneys. These alterations contribute to the development of intrarenal inflammation, interstitial renal fibrosis, and chronic kidney disease [2].

Clinically, hyperuricaemia is characterized by high sUA concentrations, defined as fasting UA levels exceeding 420 µmol/L in men and 360 µmol/L in women on two different days while adhering to a normal purine diet. A recent meta-analysis revealed that the estimated combined prevalence of hyperuricaemia in 2,277,712 individuals from mainland China was 16.4% [3]. Since this study, the prevalence of hyperuricaemia has remained at this relatively high level.

Hyperuricaemia can contribute to the sustained secretion of inflammatory factors, potentially triggering a series of inflammatory responses that may ultimately induce hyperuricaemia nephropathy. The innate immune response plays a central role in microinflammation and is closely associated with the development of hyperuricaemia nephropathy [4]. Toll-like receptor 4 (TLR4) acts as a signaling receptor mediating both innate and acquired immunity. Kidney damage is primarily caused by pro-inflammatory cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL) 6. These are induced by TLR4, which identifies ligands and initiates a complex
signaling cascade [5]. Additionally, the TLR4-mediated signaling pathway can further amplify the inflammatory response by activating the nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain-containing 3 (NLRP3) inflammasome, thereby exacerbating kidney injury.

A deeper understanding of TLR4 and the NLRP3 inflammasome will provide clearer insights into the pathogenesis of hyperuricaemia nephropathy. This paper presents a general overview of the structural composition and biological functions of TLR4 and the NLRP3 inflammasome, along with a systematic review of their relevance in the pathogenesis of hyperuricaemia nephropathy.

**Toll-like receptor 4 and hyperuricaemia nephropathy**

**Toll-like receptors (TLRs)**

The TLR family comprises a group of innate immune pattern recognition receptors, each consisting of an extracellular region, a transmembrane segment, and an intracellular region. They are named for their structural similarity in the extracellular region to the Drosophila protein Toll. TLRs are predominantly expressed on antigen-presenting cells and inflammatory cells and induce immune responses through molecular pattern receptor-mediated signaling. TLR4, a significant isoform within the TLR family, is a type I transmembrane glycoprotein. It features 16-28 leucine-rich repeats in its extracellular N-terminus, primarily recognizing pathogen-associated molecular patterns. The cytoplasmic C-terminus of TLR4 contains a highly conserved Toll/IL-1 receptor (TIR) structural domain, primarily involved in activating downstream signaling pathways [6].
TLRs recognize extracellular pathogen-associated molecular patterns (PAMPs), including carbohydrates, peptidoglycans, proteins, lipoproteins, and dextran. They mediate downstream pathways by activating transcription factors, controlling the production of pro-inflammatory cytokines and chemokines, to eliminate pathogens. In addition to exogenous (PAMPs) signals, endogenous signals from tissue damage, such as necrotic and apoptotic cells, oligosaccharides, heat shock proteins, and nucleic acid fragments, can also activate TLRs [7]. Interestingly, TLRs are now identified as key players in the pathophysiology of various inflammatory disorders, including immune-mediated illnesses, atherosclerosis, and ischemia–reperfusion-associated damage [8]. TLRs are expressed in leukocyte subpopulations and nonimmune cells, including renal cells. Recent research suggests a significant correlation between intrinsic immune activity in tissues and hyperuricaemia-related conditions, such as hyperuricaemia nephropathy. Under physiological conditions, TLR4 is abundantly expressed in renal parenchymal cells and local immune cells. It is more prominently expressed in the renal cortex than in the medulla. TLR4 expression in the renal cortex is predominantly observed in the proximal and distal tubules. Additionally, it is expressed in podocytes, human renal mesangial cells, peritubular endothelial cells, and collecting duct cells [9]. Renal TLR4 expression is modest under normal conditions but increases in response to infection and/or kidney damage. For instance, TLR4 expression increases in conditions such as lupus nephritis, unilateral ureteral obstruction, and diabetic nephropathy, as well as in renal endothelial cells, renal tubules, and infiltrating leukocytes following ischemia–reperfusion-induced damage [10-12]. These findings indicate TLR4’s critical
role in the pathophysiology of renal disorders and its potential as a promising therapeutic target for mitigating renal injury caused by these pathogenic triggers.

**Toll-like receptor 4-mediated signaling pathway**

Upon identifying external (PAMPs) or endogenous (damage-associated molecular patterns [DAMPs]) ligands, TLR4 initiates the TLR signaling cascade by forming receptor-ligand complexes. This activation triggers the recruitment of intracellular junctional proteins to the metastable TIR structural domain, subsequently mediating a series of downstream cascade reactions. These reactions lead to the activation of transcription factors, which upregulate the expression of cytokines, chemokines, growth factors, and many target genes of other inflammatory mediators.

TLR4 stimulates two primary signaling pathways, the myeloid differentiation factor 88 (MyD88)-dependent signaling pathway and the MyD88-independent signaling pathway. The MyD88-dependent signaling pathway is subdivided into the TLR4-MyD88/IL-1 receptor-associated kinase (IRAK)-mitogen-activated protein kinase (MAPK) signaling pathway and the TLR4-MyD88/IRAK-nuclear factor kappa B (NF-κB) inducible kinase (NIK)/NF-κB signaling pathway. This pathway initiates with the recruitment of TIR-associated protein (TIRAP) and MyD88 to TLR4, leading to the assembly with tumor necrosis factor receptor-associated factor-6 (TRAF6), IRAK1 and IRAK4. Upon activation, transforming growth factor-activated kinase 1 (TAK1) facilitates the phosphorylation of the nuclear factor inhibitor protein (IκB) kinase complex, comprising of IκB kinase alpha (IKKα), IKKβ, and IKKγ, which then phosphorylates the inhibitory subunit of IκB, resulting in nuclear translocation of NF-
κB and the activation of pro-inflammatory genes. Additionally, TLR4 activates the MAPK pathway, resulting in the synthesis of transcriptional activator protein-1 (AP-1), which plays a crucial role in controlling cell proliferation, differentiation, transformation, and apoptosis, and is strongly associated with inflammation, tumors and various other diseases. The MyD88-independent signaling pathway involves the recruitment of TIR-domain-containing adapter-inducing interferon (IFN)-β (TRIFs) and TRIF-related adaptor molecules (TRAMs) to TLR4 following its internalization into the endosome. Subsequently, TRAF3 activates the IFN regulatory factor-3 (IRF-3) through IKKe and TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1). This activation leads to the transcription of IFN genes, thereby promoting the activation of both the IRF-3 and NF-κB [13-15] (Figure 1).

**Toll-like receptor 4-mediated signaling pathway in hyperuricaemia nephropathy**

The key underlying mechanisms of renal abnormalities associated with hyperuricaemia are not yet fully understood. However, UA is believed to play a role in renal inflammation, intrarenal vasoconstriction, as well as in renal failure resulting from urate crystal formation. TLR4 is widely known to be a crucial molecule implicated in the pathophysiology of inflammatory disorders of the kidney. Besides its function in recognizing and combating various pathogens, TLR4 also facilitates the release of pro-inflammatory cytokines and chemokines, the latter of which encourages the local recruitment of immune cells like neutrophils and macrophages [16, 17]. Furthermore, TLR4 stimulates local kidney inflammation and fibrosis by identifying DAMPs in damaged tissues. In an experimentally induced mouse model of hyperuricaemia, UA
has been reported to induce glomerulosclerosis, tubular damage, and renal fibrosis, as well as reduced podocyte function and elevated levels of inflammatory mediators. In this model, UA led to experimental kidney injury by inducing fibroblast expansion, increased TLR4 expression, and inflammation [18]. Therefore, further in-depth studies are necessary to fully understand the specific role of the TLR4 signaling pathway in hyperuricaemia-associated renal abnormalities.

Recent studies suggest that patients with chronic kidney disease may experience oxidative stress, renal tubular damage, and intrarenal inflammation due to high sUA concentrations. Hyperuricaemia-induced kidney injury results from both urate crystal deposition and the inflammatory response triggered by UA. Notably, kidney interstitial inflammation is a key pathological mechanism in the development of hyperuricaemia nephropathy. Alongside the study of hyperuricaemia and its complications, the pro-inflammatory effects of sUA have garnered increasing attention. Studies have shown that sUA induces inflammation in vascular endothelial cells, proximal renal tubular epithelial cells, and hepatocytes, which is considered to be a key mechanism of the metabolic syndrome induced by hyperuricaemia [19]. Milanesi et al. [20] showed a significant increase in TLR4 and the pro-inflammatory cytokine monocyte chemoattractant protein 1 (MCP1) in human kidney 2 (HK-2) cells pretreated with UA. These effects were attenuated by the TLR4 antagonist resatorvid (TAK242). This suggests that UA acts as a DAMP in HK-2 cells, contributing to the induction of inflammatory and oxidative responses through TLR4. Furthermore, hyperuricaemia-related oxidative stress may further contribute to renal abnormalities, in addition to
intrarenal inflammation. Chronic hyperuricaemia has been linked to renal tubular damage and renal cortical oxidative stress in mice, primarily due to renal mitochondrial dysfunction [21]. It has been shown that hyperuricaemia increases the production of reactive oxygen species (ROS) by mediating mitochondrial calcium overload, which eventually leads to endothelial dysfunction through the mitochondrial Na⁺/Ca²⁺ exchanger [22]. Chen et al. reported that monosodium urate (MSU)-induced mitochondrial damage in cells could be prevented by reducing mitochondrial ROS, preventing the decline in mitochondrial membrane potential, and inhibiting the activation of the TLR4/NF-κB signaling pathway, thereby alleviating the MSU-induced inflammation [23].

The NLRP3 inflammasome and hyperuricaemia nephropathy

The NLRP3 inflammasome

The NLRP3 inflammasome is the most extensively studied inflammasome and is a ternary molecule within the family of nucleotide-binding oligomerization structural domain-like receptors (NLRs). The NLRP3 inflammasome consists of NLRP3, the apoptosis-associated speck-like protein (ASC) and caspase-1. Structurally, the NLRP3 inflammasome mainly consists of (1) a central nucleotide-binding oligomerization domain (NACTH) located in the middle, (2) an amino terminus that varies depending on the NLR type and consists of either a thermoprotein structural domain (pyrin domain [PYD]) or a caspase recruitment domain (CARD) for downstream bridging protein interaction, and (3) a carboxyl terminus with leucine-rich repeats (LRRs) that recognize and bind PAMPs or DAMPs. ASC, which is an articulated protein, includes a PYD at
the carboxyl terminus that binds to the PYD structural domain of NLRs and a CARD at the amino terminus that binds to the CARD structural domain of caspase-1. Caspase-1, an activated form of pro-caspase-1, cleaves cytokine precursors such as IL-1, IL-18, and IL-33, converting them into their mature forms, which are involved in the inflammatory response [24, 25]. It is generally accepted that the activation of the NLRP3 inflammasome is tightly regulated and requires two stages, initiation and activation. In the first stage, innate immune signaling through cytokine receptors (e.g., TNF receptor and/or TLR-MyD88) promotes transcription of NLRP3 and the IL-1β precursor (pro-IL-1β) via NF-κB activation. In the second stage, activation signals induce the oligomerization of the NLRP3 inflammasome, leading to ASC recruitment through PYD interactions, resulting in pro-caspase-1 activation and subsequently IL-18 and IL-1β release. Numerous agonists, such as pathogens, pore-forming toxins, environmental stimuli, and endogenous DAMPs, can activate the NLRP3 inflammasome. In addition, its activation can also result from increased potassium efflux and a rise in ROS. NLRP3 inflammasomes induce host immune responses by activating caspase-1 and the cytokines IL-1β and IL-18 [26-28].

**Regulation of the NLRP3 inflammasome signaling pathway**

Generally, the NLRP3 inflammasome activation is controlled by three distinct types of signaling pathways. These include the TLR signaling pathway, the MAPK signaling pathway, and the mammalian target of rapamycin (mTOR) signaling pathway [29-31]. The second type involves mechanisms that suppress NLRP3 activation, such as the protein kinase A (PKA), adenosine monophosphate (AMP)-activated protein kinase
(AMPK) signaling, and autophagy [32, 33]. The third type is the IFN signaling pathway, which can either enhance or inhibit NLRP3 activity depending on the physiological conditions. Each of these signaling pathways controls or inhibits the NLRP3 inflammasome activation by interfering with its assembly. The potential of targeting inflammasomes for therapeutic reasons depends on a thorough understanding of these regulatory mechanisms. Therefore, the activation mechanisms of the NLRP3 inflammasome and its associated regulatory signaling pathways are the primary focus of this review (Figure 2).

**Mitogen-activated protein kinase (MAPK) signaling pathway**

The MAPK family comprises a group of serine/threonine kinases that mediate a variety of intracellular signals related to cellular activity. The mammalian MAPK family primarily includes extracellular signal-regulated kinases (ERK) 1/2, c-Jun N-terminal kinases (JNK), and p38MAPK. The MAPK signaling pathway enhances the expression of target genes or acts directly on downstream kinases in the cytoplasm mainly through the MAPK kinase kinase (MAPKKK)/MAPK kinase (MAPKK)/MAPK triplex enzymatic reaction. This pathway is implicated in biological responses to growth factor and cytokine stimuli, as well as in recognizing abiotic stimuli such as oxidative stress, DNA damage, and osmotic imbalances, suggesting its link to inflammatory responses [34]. Previous research has demonstrated that the NLRP3-induced second signal, ROS, can activate the MAPKKK/MAPKK/MAPK pathway. Recent studies suggest that ROS can also promote NLRP3 inflammasome activation by stimulating NF-κB signaling through the MAPK (JNK/ERK/p38) pathway, thereby promoting the expression of
NLRP3 and IL-1β precursors [35]. These finding suggest that MAPK, along with downstream ROS and NF-κB, are important regulators in the activation of the NLRP3 inflammasome.

**Mammalian target of rapamycin (mTOR) signaling pathway**

The mTOR family consists of serine/threonine protein kinases that belong to the phosphatidylinositol 3-kinase-related kinase (PIKK) family. This family includes two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). As a key immune responses controller, mTOR is implicated in the differential regulation of pro- and anti-inflammatory cytokine levels [36]. A recent study has established a link between NLRP3 inflammasome activation and mTOR signaling. Rapamycin, an inhibitor mTORC1, works by targeting mTOR and inhibiting NF-κB signaling activity. This suggests that mTOR plays a role in controlling NLRP3 inflammasome activation through the NF-κB pathway [37]. Additionally, a recent published study has found that activated mTOR can induce NLRP3 inflammasome activation by increasing the production of mitochondrial ROS [38]. These findings collectively suggest that mTOR could be involved in both the initial and subsequent stages of NLRP3 inflammasome activation. Furthermore, a different study has revealed that mTORC2 can initiate the phosphorylation of serum and glucocorticoid-regulated protein kinase 1 (SGK1) in renal tubular cells. This phosphorylation stimulates epithelial sodium channels (ENaC), leading to enhanced sodium influx and potassium efflux, indicating a possible role for mTORC2 in regulating NLRP3 activation, as K⁺ efflux is a known trigger for the NLRP3 inflammasome activation [39]. In summary, these results suggest that mTOR
signaling could promote NLRP3 inflammasome activation by upregulating the expression of NLRP3, IL-1β precursors, and other components via the protein kinase B (AKT)-IKKα-NF-κB pathway, or by inducing ROS and K+ efflux. However, further research is required to fully elucidate its function in this process.

**Interferon (IFN) signaling pathway**

There are three types of IFNs: type I IFNs (IFN-α/β), type II IFNs (IFN-γ), and type III IFNs (IFN-λ). While type I and type II IFNs act on a variety of innate and adaptive immune cells, the receptor for type III IFNs is currently only found in endothelial cells [40]. Consequently, this review will focus on the roles of type I and type II IFNs in regulating NLRP3 inflammasome activation. Type I and type II IFNs bind to their respective receptors, IFN-α/β receptors (IFNARs) and INF-γ receptors (IFNRs), activating signal transducers and activators of transcription (STATs). This activation promotes the expression of interferon-stimulated genes (ISGs), which play a crucial role in controlling innate immune responses, particularly those against viral infections [41]. An important mechanism by which IFN signaling regulates innate immunity is by regulating the activation of inflammasomes, especially the NLRP3 inflammasome. A recent study clarified the relationship between IFN signaling and the NLRP3 inflammasome. It found increased protein levels of ASC, NLRP3 and caspase-1 in stimulated myotubes, and increased levels of IL-1β in cell culture supernatants under IFN-γ-induced inflammatory conditions. These findings indicate that the NLRP3 inflammasome is activated in IFN-treated myotubes [42].
The NLRP3 inflammasome-mediated signaling pathway in hyperuricaemia nephropathy

Several studies indicate that elevated UA levels are associated with renal inflammation, tubular damage, tubulointerstitial fibrosis, UA kidney stones, chronic interstitial nephritis, and consequently hyperuricaemia nephropathy, which may progress to chronic kidney disease (CKD) or end-stage renal disease (ESRD). The hyperuricaemia nephropathy inflammatory phenotype is characterised by elevated levels of cytokines, chemokines, and adhesion molecules in renal vascular endothelial cells. This chronic inflammatory response in the vascular endothelium leads to the production of inflammatory mediators, which enhance vascular permeability, promote endothelial cell apoptosis, and contribute to neointimal formation [43]. The NLRP3 is a key player in sensing danger signals in the cytosol, including PAMPs and DAMPs. This sensing leads to the activation of caspase-1, IL-1β, IL-18, and other cytokines, stimulating an inflammatory cascade critical in hyperuricaemia nephropathy. UA itself acts as a DAMP, aberrantly activating the immune system and the NLRP3 inflammasome, thereby inducing tissue damage and diseases such as gout and chronic kidney injury [44, 45].

Previous research has demonstrated that elevated sUA levels are a risk factor for the development of nephropathy. Chronic kidney injury due to hyperuricaemia commonly manifests as tubulointerstitial inflammation and damage, accompanied by increased macrophages and T-cells infiltration in the tubulointerstitium. In a study by Wu et al. [2] disruption of UA oxidase in rats led to a spontaneous and sustained increase in sUA levels in vivo. This resulted in enhanced interstitial fibrosis, macrophage infiltration,
elevated NLRP3 and IL-1β expression, and the stimulation of numerous cellular signaling pathways associated with autophagy, including the AMPK, p38MAPK, ERK, and JNK pathways. Interestingly, the phosphoinositide 3-kinase (PI3K) inhibitor 3-methyladenine (3-MA), which inhibits autophagy, was found to prevent renal injury onset and reduce renal fibrosis, macrophage infiltration, and the production of NLRP3 and IL-1β in injured kidneys. These findings suggest that hyperuricaemia-induced autophagy and NLRP3-dependent inflammation contribute to kidney injury, tubular damage, and renal fibrosis. Xanthine oxidase (XO), a major source of ROS, reacts with xanthine and hypoxanthine to form UA. Hyperuricaemia leads to excessive oxidative stress, potentially promoting hyperuricaemia nephropathy. Based on this, xanthine oxidoreductase (XOR) inhibitors, such as febuxostat, might offer protection against renal disease progression. In support of this, a study demonstrated that febuxostat protected the kidneys from renal ischemia-reperfusion injury by inhibiting oxidative stress and delaying glomerular hypertrophy and tubulointerstitial fibrosis [46].

Similar to oxidative stress, inflammation plays a crucial role in all stages of hyperuricaemia nephropathy. Vazirpanah et al. [47] found that isolated monocytes from gout patients exhibited increased mTOR pathway expression. Using real-time imaging, they observed that these monocytes initiated cell death and released multiple pro-inflammatory cytokines upon encountering MSU crystals. This indicates that mTOR inhibition may emerge as a new therapeutic target in treating hyperuricaemia nephropathy.
Function of the TLR4/MyD88 signaling pathway and the NLRP3 inflammasome in hyperuricaemia nephropathy

Recently, it was discovered that innate immunity is related to various metabolic disorders. It has been reported that innate and adaptive immune responses play a role in the inflammatory mechanisms of cardiovascular diseases such as atherosclerosis and hypertension [48], and experimental studies have already demonstrated an association between immune dysregulation and cardiac, vascular, and renal alterations in hypertensive patients [49]. Indeed, renal epithelial cells have immunological privileges, and they are encircled by a dense network of immune cells, creating a setting for interactions between immune cells and renal tubular cells. It is now believed that uric acid is not only involved in causing chronic interstitial nephritis through the deposition of urate crystals in the renal tubules and renal interstitium, but more importantly, it is also involved in promoting glomerulosclerosis and fibrosis in interstitial cells by impairing vascular endothelial function, stimulating vascular smooth muscle cell proliferation, promoting inflammatory responses and immune responses, activating the renin-angiotensin-aldosterone system, altering haemodynamics, and increasing systemic blood pressure and intraglomerular pressure, among other multiple effects [50].

During the NLRP3 inflammasome initiation, UA activates TLR4 via the bridging protein MyD88, and ultimately NF-κB is activated to enhance pro-IL-1β and NLRP3 expression. TLRs, including TLR2, TLR4, TLR6 and their coreceptor CD14, are integral components of the receptor complex that activates renal cells via uric acid,
which can initiate the typical inflammasome pathway by activating the TLR4/MyD88/NF-κB signaling pathway, resulting in the production of cytokines and chemokines [51]. During the activation step, uric acid leads to lysosomal damage and/or mitochondrial reactive oxygen species production, which triggers the formation of the NLRP3 inflammasome complex, leading to Caspase-1 activation, and IL-1β production [52]. In addition, secreted IL-1β induces renal inflammasome initiation via IL-1R/MyD88 signaling [53]. Therefore, it is suggested that the vicious cycle of TLR4/MyD88 signaling activating the NLRP3 inflammasome may lead to chronic inflammation and the deterioration of renal function in hyperuricaemia kidneys. Xiao et al. [54] provided further evidence that soluble UA regulates innate immune damage via TLR4-dependent NLRP3 inflammasome formation, which causes Caspase-1 activation and the production of IL-1β and ICAM-1 in human primary renal PTECs. Numerous disorders have been linked to innate immunity, which means that many of the pro-inflammatory effects of UA may be mediated by innate immune activation through TLR4. Consequently, focusing on innate immune pathways may offer an alternate method of treating inflammatory illnesses. In a different experiment, Xiao et al. [55] further elucidated the connection between TLR4/NLRP3 and hyperuricaemia nephropathy inflammation, and found that sUA significantly enhanced TLR4, NLRP3, and IL-1β expression, while the TLR4 inhibitor TAK242 significantly blocked soluble uric acid-induced NLRP3 and IL-1β upregulation, suggesting that sUA may play a pathological role in renal tract injury induced by hyperuricaemia. Thus, the ability of TLR4 and NLRP3 to respond to cellular stress and stimuli makes them key factors in
maintaining inflammation in aseptic inflammatory diseases such as hyperuricaemia nephropathy, and TLR4 and the NLRP3 inflammasome initiate the transcription of inflammatory factors that play important roles in the pathogenesis of hyperuricaemia nephropathy providing new ideas and strategies for the prevention and treatment of hyperuricaemia nephropathy.

**TLR4 and the NLRP3 inflammasome-targeted therapeutic strategies for hyperuricaemia nephropathy**

Hyperuricaemia nephropathy is a metabolic disease with a high disability rate. Although anti-inflammatory medications (NSAIDs, colchicine, and glucocorticoids) have been used to treat hyperuricaemia, their usage has been limited due to negative effects (significant hepatotoxicity and hyperkalaemia). Hence, it is imperative to develop pharmaceutical agents that are both safe and efficacious in treating hyperuricaemia nephropathy. As previously declared, TLR4 and NLRP3 inflammasomes play a significant role in hyperuricaemia kidney injury; and thus, in recent years, the scientific community has been attempting to develop drugs that specifically inhibit TLR4 and NLRP3 inflammasome for the treatment of hyperuricaemia nephropathy. In this paper, we provide an overview of the treatment strategies involving TLR4 and NLRP3 inflammasome and classify these medications into three groups: the extracts from natural products, traditional Chinese medicine formula and novel inhibitors.

The extracts from natural products: Several TLR4 or NLRP3 inflammasome inhibitors have been developed to reduce renal inflammation by inhibiting TLR4 or NLRP3
inflammasome activity. Dioscin is effective in removing dampness, clearing turbidity, dispelling wind and relieving pain. According to the experiment by Han et al. [56], dioscin lower blood uric acid levels and may successfully cure hyperuricaemia by suppressing pro-inflammatory cytokine production, blocking the TLR4/NF-\(\kappa\)B signaling pathway, and inhibiting NLRP3 activation. Furthermore, hesperetin, a natural flavonoid which has several biological activities, decreases uric acid by blocking xanthine oxidase activity and protein expression, tampering with the TLR4-NLRP3 inflammasome signaling pathway [57]. Lagotis brachystachya maxim is an herb commonly used in traditional Tibetan medicine, which is mainly used to alleviate local inflammation in Tibet. According to Zhu et al. [58], lagotis brachystachya maxim extract decreases hyperuricaemia by decreasing uric acid synthesis and raising uric acid excretion, as well as by blocking the TLR4/MyD88/NLRP3 inflammasome signaling pathway. To sum up, these natural constituents have nephroprotective properties, but only in animals. Consequently, it is necessary to investigate the pharmacological and safety uses of natural constituents in human medicine.

Traditional Chinese medicine formula: Several widely recognised Chinese herbal formulas have been demonstrated to reduce renal inflammation and dysfunction by regulating the NLRP3 inflammasome or TLR4. Wu Ling San has the efficacy of improving kidney function and diuresis and has a protective effect on the kidneys of fructose-induced hyperuricaemia mice, which can significantly reduce the levels of serum uric acid, creatinine and urea nitrogen, and increase the fraction of uric acid excretion in fructose-induced mice. The molecular mechanism of its specific action
may be related to the inhibition of TLR4/MyD88 signaling and the NLRP3 inflammasome activation, which reduces the production of IL-1β in high fructose-induced hyperuricaemia mice [59]. Besides, researches have shown that the herbs in Shizhifang are secure and beneficial in decreasing uric acid and safeguarding renal function [60, 61]. According to a research by Zhou et al. [62], Shizhifang can stop renal tubular inflammation and the advancement of uric acid-induced renal damage by inhibiting the apoptosis of renal tubular epithelial cells by targeting NLRP3.

Novel inhibitors: MiR-146a is implicated not only in innate immune modulation, but also in carcinogenesis and inflammatory development. Research has shown that TRAF6 and IRAKI are significant miR-146a target genes [63]. At the same time, as critical components of the TLR4 signal transduction pathway, they play important roles in activating downstream signaling pathways [64]. Chen et al. [65] demonstrated that miR-146a significantly decreased the gouty joint swelling index, joint dysfunction index, and joint inflammation index. Additionally, in synovial tissues, miR-146a showed significantly lower expression levels of TLR4 and MyD88 and related inflammatory factors (TNF-α, IL-1β, and IL-6), which attenuated joint inflammation in acute arthritis in rats. These findings may suggest that miR-146a represents a new therapeutic target for hyperuricaemia nephropathy. Moreover, it has been extensively reported that the new adipokine C1 q-Tumour necrosis factor-related protein 3 (CTRP3) has key anti-inflammatory, anti-fibrotic, and anti-apoptotic effects. Furthermore, it's possible that CTRP3 protects against kidney diseases [66]. Zhang et al. [67] found that CTRP3 improves endothelial cell morphology and apoptosis in hyperuricaemia through
suppressing TLR4-mediated inflammatory responses and decreasing oxidative stress.

CONCLUSION

Increasing amounts of data point to hyperuricaemia as a separate risk factor for the onset and progression of CKD. The hyperinflammatory state of hyperuricaemia nephropathy is highly correlated with TLR4-mediated regulation of NLRP3 inflammasome induced immune and inflammatory responses through crosstalk between its signaling pathways. TLR4 and NLRP3 inflammasome activation promotes the maturation and release of Caspase-1 and downstream cytokines such as IL-1β and IL-18, triggering a persistent pro-inflammatory state that is important for the continued progression of hyperuricaemia nephropathy. TLR4 and the NLRP3 inflammasome have emerged as attractive therapeutic targets, and regulating inflammatory responses by modulating TLR4, the NLRP3 inflammasome and downstream signaling to reduce their expression or inhibit their activity is expected to be a new strategy for the treatment of hyperuricaemia nephropathy. Despite the wealth of information supporting the contribution of TLR4 and the NLRP3 inflammasome to the pathophysiology of hyperuricaemia nephropathy, therapeutic interventions targeting these pathways have not been successfully applied clinically; therefore, targeted drug studies of TLR4 and the NLRP3 inflammasome will be important in treating the complications of hyperuricaemia.

Given the significance of therapy timing, there is an urgent need for targeted therapies, which are important for reducing or mitigating the disease and improving the prognosis. In animal experiments, according to the mechanism of NLRP3 inflammasome and
TLR4 activation, compounds and molecules that interfere with these processes may reduce the production of NLRP3 inflammasome and TLR4, therefore attenuating hyperuricaemia renal damage. Significantly, however, the pharmacokinetic profile and limited safety profile of this class of inhibitors restrict their clinical use. Apart from the current understanding on TLR4 and NLRP3 inflammasome, there are still numerous of significant unanswered issues. What additional mechanisms, for instance, are in charge of TLR4 and the NLRP3 inflammasome activation? Apart from the mTOR, IFN, and ROS pathways, do TLR4 and NLRP3 also regulate additional vital signaling pathways? And, what about TLR4 and NLRP3 inflammasome inhibitors' possible impacts on peripheral immune function, and how would these affect immune system defence mechanisms? What about the best time and dose to provide these inhibitors after a diagnosis of hyperuricaemia? The answers to these issues will provide new clues to our understanding of the mechanisms behind renal damage caused by hyperuricaemia and enhance our comprehension of the roles played by TLR4 and NLRP3 in hyperuricaemia nephropathy. Therefore, active development of newer FDA-approved therapies is imperative for the future treatment of hyperuricaemia nephropathy. In light of this, we propose TLR4 and NLRP3 inflammation as viable therapeutic targets for hyperuricaemia nephropathy.

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Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.
REFERENCES


FIGURE 1. The TLR4 signaling pathway. Upon interaction with LPS, TLR4 forms homodimers and initiates signaling through both the MyD88-dependent and MyD88-independent pathways. The MyD88-dependent pathway starts with the recruitment of TIRAP and MyD88 to TLR4, which then assembles with TRAF6, IRAK1 and IRAK4. Once activated, TAK1 facilitates the phosphorylation of the IKK (comprising IKKα, IKKβ, and IKKγ), which phosphorylates the inhibitory subunit of IκB. This leads to the nuclear translocation of NF-κB and the activation of pro-inflammatory genes. Additionally, TLR4 activates the MAPK pathway, resulting in the synthesis of transcriptional AP-1, a crucial factor for controlling cell proliferation, differentiation, transformation, and apoptosis, and strongly linked to
inflammation, tumors, and various other diseases. The MyD88-independent signaling pathway involves the recruitment of TRIF and TRAM to TLR4 following its internalization into the endosome. The pathway leads to TRAF3 activation, which subsequently induces the transcription of IFN genes, thereby promoting the activation of IRF-3 and NF-κB. The figure was created with BioRender.com. TLR4: Toll-like receptor 4; LPS: Lipopolysaccharide; MyD88: Myeloid differentiation factor 88; TIRAP: TIR-associated protein; TIR: Toll/IL-1 receptor domain; IL-1: Interleukin 1; TRAF: Tumor necrosis factor receptor-associated factor; IRAK: IL-1 receptor-associated kinase; TAK1: Transforming growth factor-activated kinase 1; IKK: IκB kinase; IκB: Nuclear factor inhibitor protein; NF-κB: Nuclear factor kappa B; MAPK: Mitogen-activated protein kinase; AP-1: Activator protein-1; TRIF: TIR-domain-containing adapter-inducing interferon-β; TRAM: TRIF-related adaptor molecule; IFN: Interferon; IRF-3: Interferon regulatory factor-3; TAB: TAK1 binding protein; RIR1:
Activation of the NLRP3 inflammasome requires two distinct signals. The first signal involves the upregulation of NLRP3 and pro-IL-1 transcription by activating the signaling pathways controlled by TLRs-NF-κB and IFN. The second signal, essential to promote the formation of the inflammasome and the activation of caspase-1, is the potassium ion (K+) efflux and ROS production. These processes are mediated by the mTORC1/2 complex and apoptosis signaling pathways. Once activated, caspase-1 cleaves pro-IL-1β into its mature form, IL-1β, and also cleaves GSDMD, leading to the formation of pores in the plasma membrane and initiating pyroptosis. The figure was created with BioRender.com. NLRP3: Nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3; IL: Interleukin; TLRs: Toll-like receptors; NF-κB: Nuclear factor kappa B; IFN: Interferon; ROS: Reactive oxygen species; mTORC: Mammalian target of rapamycin complex; GSDMD: Gasdermin D; JAK: Janus.
kinase; STAT1: Signal transducer and activator of transcription; P: Phosphorylation; MyD88: Myeloid differentiation factor 88; TIRAP: TIR-associated protein; TIR: Toll/IL-1 receptor domain; TRAF6: Tumour necrosis factor receptor-associated kinase 6; TAK1: Transforming growth factor-activated kinase 1; TAB: TAK1 binding protein; IKK: IκB kinase; MAPKs: Mitogen-activated protein kinases; p38: p38 mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; ERK: Extracellular signal-regulated kinase; mtROS: Mitochondrial reactive oxygen species; TXNIP: Thioredoxin-interacting protein; ASC: Apoptosis-associated speck-like protein; GSDMD-NT: Gasdermin D N-terminal.