

NLRP3 inflammasome: A therapeutic option for kidney disease?

El inflammasoma NLRP3: ¿Es realmente una opción terapéutica para la enfermedad renal?

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ABSTRACT

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Objective To determine if considering inflammasome NLRP3 as a treatment option for kidney disease is possible.

Methods Literature review related to NLRP3 inflammasome structure, biological function and relationship with renal disease and others (hypertension, diabetes, gout, atherosclerosis, amyloidosis, Alzheimer's disease); the systematic review was made searching in the databases PubMed and SciELO for the following terms: "The NLRP3 inflammasome therapeutic for kidney disease", "NLRP3 inflammasome in kidney disease" in PubMed, and "Inflammasome" for Scielo.

Results 146 documents were found, although only 34 matched the working hypothesis concerning the NLRP3 inflammasome as a central component of various diseases in humans, with potential therapeutic use. The NLRP3 inflammasome is responsible for the maturation of inflammatory pro-interleukin IL-1 β and IL-18, which can be triggered by aggregated or crystalline materials (particles), and by various microorganisms and toxins derived from these; however, the way how activation mechanisms work is not completely clear.

Conclusions Research on new therapies that focus on removing or inhibiting inflammasome components, both individually and together, is proposed.

Key Words: Inflammation, inflammasomes, chronic renal diseases, hypertension, interleukins (*source: MeHS, NLM*).

RESUMEN

Objetivo Determinar si el inflammasoma NLRP3 puede considerarse como opción de tratamiento para la enfermedad renal.

Métodos Con el fin de encontrar bibliografía relacionada con la estructura del inflammasoma NLRP3, su función biológica y su relación con la enfermedad renal y otras (hipertensión, diabetes, gota, aterosclerosis, amiloidosis, enfermedad de Alzheimer), se realizó una revisión sistemática en dos bases de datos (PubMed y SciELO) con los términos: "NLRP3 inflammasome therapeutic for kidney disease" y "NLRP3 inflammasome in kidney disease" en PubMed, e "inflammasome" en SciELO.

Resultados Se encontró un total de 146 documentos, de los cuales solo 34 concuerdan con la hipótesis de trabajo desarrollada con relación al inflammasoma NLRP3 como componente central de diversas enfermedades en seres humanos y con potencial uso terapéutico. El inflammasoma NLRP3 es responsable de la maduración de la interleucina inflamatoria pro-IL-1 β y IL-18, la cual puede darse por causa de materiales agregados o cristalinos (partículas), y por diversos microorganismos y toxinas derivadas de los mismos; sin embargo, los mecanismos de activación de este proceso siguen sin ser claros en la actualidad.

Conclusiones Se propone estudiar nuevas terapias que se centren en la eliminación o inhibición de los componentes inflammasoma, de manera individual y conjunta.

Palabras Clave: Inflamación, inflammasomas, insuficiencia renal crónica, interleucinas, hipertensión (*fuentes: DeCS, BIREME*).

A qualitative systematic review of documents was performed, only in English, in the PubMed and Scielo databases, without using any quality assessment model for the treatment of the information found. Of the 146 works selected, only 42 were related to the assembly and activation of the NLRP3 inflammasome: 27 were about pathophysiological mechanisms of renal damage and NLRP3 inflammasome, 12 about oxidative stress, 9 about autoimmunity, and 56 about other diseases; the search was restricted to the period 2002-2015. 34 documents related directly to the working hypothesis — activation or assembly NLRP3 inflammasome as a therapeutic target in kidney disease, hypertension, diabetes, and related diseases— were included.

Inflammation is a control program preserved evolutionarily and derived from host defenses to deal with infections and tissue damage. Innate immune cells such as macrophages, mast cells and dendritic cells, as well as circulating leukocytes, are able to recognize the invasion by microorganisms and cell damage through a set of receptor proteins called pattern recognition receptors (PRRs) (1-6). PRRs are plasma membrane-anchored or intracellular receptors that detect microbe-associated molecular patterns (MAMPs) which derive from microorganisms, and damage-associated molecular patterns (DAMPs) which derive from endogenous danger signals (7).

Activation and assembling of PRRs in large supra-molecular complexes (e.g. inflammasomes) initiate the signaling cascades that allow the release of cytokines, chemokines, as well as the recruitment of immune cells into the tissue damaged (8-9).

The NLRP3 inflammasome is the best characterized of these multiprotein complexes; it contains leucine-rich repeats (LRR) in its N-terminal end, a highly conserved central nucleotide-binding domain (NACHT by its acronym, also known as nucleotide oligomerization domain or NOD), and a PYD domain in its C-terminus end (10-11). This receptor is the most important PRR involved in the assembly and activation of inflammasome (12).

After activation, NLRP3 protein oligomerizes and interacts with two additional proteins, ASC protein (which contains a caspase recruitment domain and a pyrene domain) and procaspase-1. As a result of this interaction, caspase-1 protein is activated (13). The processing of caspase-1 is essential for proteolytic cleavage and secretion of inflammatory pro-interleukins 1 β (pro-IL1 β) and 18 (proIL-18). Under certain conditions, activation of caspase-1 generates pyroptosis, a form of inflammatory cell death similar to apoptosis and necrosis (14). One relevant aspect of NLRP3 inflammasome activation is that signaling and the mechanisms for activation and

assembly by various exogenous and endogenous noxious stimuli are not fully elucidated yet.

On the other hand, the defective activation and disproportionate action of the NLRP3 inflammasome relates to the etiology of various diseases and autoinflammatory syndromes such as hereditary periodic fever syndrome, the Muckle-Wells syndrome (15), septic shock induced by lipopolysaccharide (16), gout (17), type 2 diabetes (18), metabolic syndrome (19), hypertension (2), atherosclerosis (19), amyloidosis (20) and Alzheimer's disease (21).

Hypotheses

One important question in nephrology is whether kidney diseases are related directly or indirectly to the NLRP3 inflammasome and, if so, how and where the problem begins, since many authors conclude that renal pathologies have, as common characteristics, the extravasation and infiltration of inflammatory cells, as well as the expression and secretion of the cytokines IL-1 β and IL-18 in the kidney tissue (14). Bakker (2014) pointed out that chronic kidney disease is irreversible and is characterized by tubulointerstitial inflammation, fibrosis and glomerulosclerosis, which are associated with hyperuricemia; it is further known that the latter causes NLRP3 inflammasome activation (22).

Our research group has proposed that the expression of heat shock proteins (HSPs) and the concentration of oxygen and nitrogen reactive species (ROS and RNS respectively) are increased in the kidneys of patients (3, 23-24). On the other hand, the monosodium urate crystals resulting from hyperuricemia are effectors of the NLRP3 activation (17).

Researchers have speculated about the differences in signaling mechanisms caused by the NLRP3 inflammasome to direct infiltration of inflammatory cells and epithelial-mesenchymal transformation in the renal tubule. This process is associated with tubular atrophy and progressive interstitial fibrosis in kidney tissue, which apparently runs NLRP3 by stimulation with TGF- β in different renal compartments (2).

Wree (2014) previously showed that the NLRP3 inflammasome can be expressed constitutively and can also be the cause of damage to the liver (25). This activation results in abnormal development of cellular stress and, therefore, will generate a positive feedback, which will increase the degree of the inflammatory process; this raises the question of whether this constitutive expression could occur in other organs. It is necessary to devise new strategies to address, from different perspectives, the study of inflammasomes, including genetic and epigenetic analysis of each case, to direct appropriate anti-inflammatory therapies in a personalized manner.

Another important function of NLRP3, besides acting as a sensor of cell damage and activating an inflammatory response in the cell cytoplasm, is its dual subcellular location; it is located in the cytosol and is also associated with other organelles (e.g. the nucleus), even in differential locations of T cell subsets. A possible explanation for the difference in the activity of TH1 and TH2 cells could be the subcellular localization of NLRP3. Cytoplasmic localization of NLRP3 might promote inflammasome assembly, while nuclear location might favor the transcriptional function of the inflammasome (26), which is reflected on the results of the work of Bruchard (27), who found that NLRP3 expression in CD4⁺ T cells, specifically, supported type 2 T helper (TH2) transcriptional programs in a cell-intrinsic manner.

NLRP3 positively regulated a TH2 program, although this was not the case of the inflammasome adaptor ASC nor of caspase-1. In TH2 cells, NLRP3 bounded the *Il4* promoter and transactivated it along with the transcription factor IRF4. *Nlrp3*-deficient TH2 cells supported melanoma tumor growth in an IL-4-dependent manner and also promoted asthma-like symptoms. These results demonstrate the ability of NLRP3 to act as a key transcription factor in TH2 differentiation.

Together, these observations on the effects and actions of the NLRP3 inflammasome on innate immunity and tissue damage make possible to see that the role of this complex goes beyond a simple proteolytic processing, proinflammatory cytokines activation, transcriptional functions, and pyroptosis induction.

One of the conclusions after our review of the literature on renal diseases is that basic and applied research in the renal field in relation to inflammasome should now focus on whether the therapeutic approach or the pharmacological inhibition of NLRP3 are sufficient for treatment, even for reversing kidney problems, in order to be closer to ensure a better quality of life for patients.

Table 1 shows the five compounds that have the ability of inhibiting NLRP3 inflammasome activation by directly interfering with the inflammasome or this assembly. There are four exogenous (synthetic) compounds that inhibit inflammasome-NLRP3, namely, Bay 11-782 (28), 3, 4-methylenedioxy- β -nitrostyrene (MNS) (29), MCC 950 (34) and dimethyl sulfoxide (DMSO) (30), although these agents have limited potency and are non-specific.

In this sense, MCC950 (small-molecule inhibitor of the NLRP3 inflammasome) blocked canonical and non-canonical NLRP3 activation at nanomolar concentrations. The half maximal inhibitory concentration (IC_{50}) of MCC950 was approximately 7.5 nM in mouse bone marrow-derived macrophages, which is similar to

human monocyte-derived macrophages (IC_{50} = 8.1 nM). The secretion of LPS-dependent tumor necrosis factor- α was not impaired by MCC950, which demonstrates that the inhibition of IL-1 β secretion was specific. MCC950 inhibited specifically the activation of NLRP3, but not of the AIM2, NLRC4 or NLRP1 inflammasomes. MCC950 reduced interleukin-1 β (IL-1 β) production *in vivo*, and attenuated the severity of experimental autoimmune encephalomyelitis (31).

Table 1. Inhibitory compounds of the NLRP3 inflammasome

Inhibitor	Nature	Mechanisms of action	Reference
BHB	Endogenous	It acts by preventing K ⁺ efflux and reducing ASC oligomerization and speck formation.	33
MNS	Exogenous	MNS specifically prevented NLRP3-mediated ASC speck formation, as well as oligomerization, without blocking potassium efflux induced by NLRP3 agonists.	29
DMSO	Exogenous	It inhibits ASC pyroptosome formation via NLRP3 inflammasome activators.	30
Bay 11-7082	Exogenous	Inhibitor of the ATPase activity of NLRP3 inflammasome, and also inhibitor of the protease activity of caspase-1.	28
MCC950	Exogenous	It could prevent NLRP3-induced ASC oligomerization.	34

In this sense, MCC950 (small-molecule inhibitor of the NLRP3 inflammasome) blocked canonical and non-canonical NLRP3 activation at nanomolar concentrations. The half maximal inhibitory concentration (IC_{50}) of MCC950 was approximately 7.5 nM in mouse bone marrow-derived macrophages, which is similar to human monocyte-derived macrophages (IC_{50} = 8.1 nM). The secretion of LPS-dependent tumor necrosis factor- α was not impaired by MCC950, which demonstrates that the inhibition of IL-1 β secretion was specific. MCC950 inhibited specifically the activation of NLRP3, but not of the AIM2, NLRC4 or NLRP1 inflammasomes. MCC950 reduced interleukin-1 β (IL-1 β) production *in vivo*, and attenuated the severity of experimental autoimmune encephalomyelitis (31).

Renal inflammation, fibrosis and elevated blood pressure induced by 1K/DOCA/salt treatment in mice depend on inflammasome activity, stressing the inflammasome/IL-1 β pathway as a potential therapeutic target in hypertension. Additionally, MCC950 (at a concentration of 10 mg kg⁻¹d⁻¹, s.c.) reversed hypertension in the animals treated with 1K/DOCA/salt (32).

Other endogenous compounds block the activation of NLRP3 inflammasome. Youm (33) reports that one of such compounds is ketone body, β -hydroxybutyrate (BHB) — but not the structurally related acetoacetate or butyrate—, which specifically inhibits NLRP3 inflammasome activation and downstream cytokine production by numerous known NLRP3 activators in mouse bone marrow-derived macrophages and human monocytes *in vitro*.

Dose-dependently BHB inhibited $\text{IL-1}\beta$ and IL-18 secretion, without significantly affecting tumor necrosis factor (TNF- α) production, as demonstrated in culture supernatants of human monocytes stimulated with LPS (1 $\mu\text{g/mL}$) for 4 hours, in the presence of an increasing concentration of BHB (1, 10 and 20 mM). At millimolar concentrations BHB blocks the NLRP3 inflammasome without undergoing oxidation in the tricarboxylic acid cycle, and independently of uncoupling protein-2 (UCP2), sirtuin-2 (SIRT2), G protein-coupled receptor GPR109A, or hydrocaboxylic acid receptor 2 (HCAR2). When administered in complex to mice at physiological concentrations with nanolipogens that improve bioavailability, β -hydroxybutyrate blocked NLRP3 inflammasome activation in response to monosodium urate (MSU) crystals, which is the causative agent of gout (33-34).

Up to date, the only clinically available therapeutics for NLRP3-driven autoinflammatory diseases are antibodies targeting $\text{IL-1}\beta$ signaling, which are not specific for NLRP3 activity. Unlike anakinra, rilonacept, and canakinumab, both MCC950 (synthetic) and BHB (endogenous) act as specific inhibitors of NLRP3 and do not affect Toll-like receptors signaling (TLR) or other inflammasome-forming NLRs (34).

DISCUSSION

NLRP3 inflammasome activation results in the release of pro-inflammatory interleukins. Several authors have demonstrated the presence of these interleukins in organs with inflammasome hyperactivation caused by intrinsic or extrinsic damage, for which kidney disease is not the exception; however, inflammasome activation has not been proved to be the cause in the light of an experimental model. Therefore, studying new therapies that focus on removing or inhibiting inflammasome components, both individually and together, is proposed in order to develop the hypothesis raised here.

The involvement of inflammasome in human disease has incited efforts to identify potent and specific ways to interfere with NLRP3 activation in the context of auto-inflammatory diseases, including other diseases such as obesity, diabetes and hypertension [▲]

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