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Nonpharmacological, Biomechanical Approaches to Control Inflammation in Acute Kidney Injury

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Abstract
Inflammation is broadly recognized as an important factor in the pathogenesis of acute kidney injury (AKI), but pharmacological approaches to alleviate inflammation in AKI have been without success in clinical trials. Neuromodulation by nonpharmacological methods is emerging as a novel therapeutic strategy to treat inflammatory diseases. Recently, our group and others have demonstrated that vagus nerve stimulation and pulsed ultrasound ameliorated inflammation via the cholinergic anti-inflammatory pathway (CAP) in various animal models, including renal ischemia-reperfusion injury. Delineating the precise mechanisms by which these methods activate the CAP and ameliorate inflammation is mandatory for the broad clinical application in the future. Novel techniques, such as optogenetics, are expected to elucidate these complex mechanisms.

Introduction

Acute kidney injury (AKI) is a serious clinical disorder because it is strongly associated with high mortality and AKI episodes may lead to chronic kidney disease and end-stage renal disease. Supportive therapy is currently the only treatment strategy for AKI, and no drug (therapeutic or preventive) is approved for AKI treatment in humans [1]. Despite our understanding of mechanisms responsible for inflammation in the pathogenesis of AKI, pharmacological approaches in clinical trials to alleviate inflammation have been without success [2]. Thus, other approaches are eagerly sought to ameliorate inflammation in AKI. Among these approaches, neuromodulation by nonpharmacological

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methods is emerging as a powerful approach to counter inflammation, as many studies have revealed neural pathways that regulate inflammation and immunity [3]. In this review, we discuss several nonpharmacological approaches to modulate the neuroimmune axis and alleviate inflammation as a promising treatment strategy against AKI.

The Inflammatory Reflex

In 1995, Watkins et al. [4] reported that subdiaphragmatic vagal transection abolished hyperthermia following intraperitoneal injection of interleukin-1β, demonstrating that peripheral inflammation activates the afferent vagus nerve to initiate a fever response. Subsequently, Tracey et al. [3] found that a small amount of CNI-1493 (a potent anti-inflammatory agent), which had been previously demonstrated by Kramer et al. [5] to ameliorate the distant lung inflammation during AKI through systemic administration, administered via the intracerebroventricular route decreased not only the level of brain tumor necrosis factor (TNF) but also that of plasma TNF, which originates mostly from the spleen, in lipopolysaccharide-treated rats [6]. They also demonstrated that severing the vagus nerve abolished the decrease in plasma TNF level by CNI-1493 administration and that only electrical stimulation of the vagus nerve was enough to reduce plasma TNF. This result suggested that signals descend from the brain through the vagus nerve to the spleen to block inflammation. Thus, the principle forming "the inflammatory reflex" is that the afferent vagus nerve senses peripheral inflammatory stimulation and the signal is transmitted to the efferent vagus nerve to abrogate inflammation in the peripheral.

The inflammatory reflex is characterized by several mechanisms summarized in online supplementary Figure 1 (see www.karger.com/doi/10.1159/000477218). (1) The afferent vagus nerve is stimulated by inflammatory products, including cytokines, damage-associated molecular patterns, and pathogen-associated molecular patterns, through cytokine receptors and pattern-recognition receptors expressed in the vagus nerve [7]. (2) The nerve activity is transmitted to the brain, resulting in the activation of the efferent vagus nerve. (3) The signal is relayed to the splenic nerve [8]. (4) Norepinephrine released from the splenic nerve interacts with β2-adrenergic receptors in choline acetyltransferase-positive T cells in the spleen, causing acetylcholine (ACh) release from these T cells [9]. (5) ACh interacts with α7 nicotinic ACh receptors (α7nAChRs) in macrophages residing close to choline acetyltransferase-positive T cells, resulting in suppressed proinflammatory cytokine production and inflammation [10]. The efferent arm of the inflammatory reflex is termed the cholinergic anti-inflammatory pathway (CAP) [3].

Nonpharmacological Strategies to Control Inflammation in AKI

Vagus Nerve Stimulation

We recently demonstrated that vagus nerve stimulation (VNS) 24 h before renal ischemia dramatically protected the kidney from renal ischemia-reperfusion injury (IRI) [11]. It is interesting that stimulating the central end (afferent VNS) or the peripheral end of the cut vagus nerve had comparable renoprotective effects against the stimulation of the intact vagus nerve. The mechanism responsible for the protective effect of afferent VNS is likely to be more complex than a vago-vagal response because stimulating the afferent limb was still protective when the contralateral vagus was anesthetized and presumably nonconductive. These unknown pathways in the brain following afferent VNS clearly merit further investigation. VNS was also reported to be beneficial to decrease chronic allograft nephropathy with reduced immune cell infiltration [12].

Many clinical trials are underway to translate the protective effect of VNS into treatment for patients with inflammatory and autoimmune diseases (110 studies on ClinicalTrials.gov using "vagus nerve stimulation"; https://clinicaltrials.gov [accessed March 3, 2017]). In patients with rheumatoid arthritis, vagus nerve activity was significantly lower than that in controls [13]. In addition, serum high mobility group box 1 levels and vagus nerve activity showed a significant inverse association. These data suggest that decreased CAP activity is associated with disease activity and that activation of CAP may be beneficial in these patients. The first clinical trial that used an implanted electronic device in patients with refractory rheumatoid arthritis was recently reported [14]. VNS significantly inhibited TNF production and improved disease severity for up to 84 days. The effectiveness of VNS has also been demonstrated in patients with Crohn's disease [15, 16]. These results showed that VNS can reduce inflammation in humans, offering a promising view of the usefulness of VNS for other inflammatory diseases, including AKI.

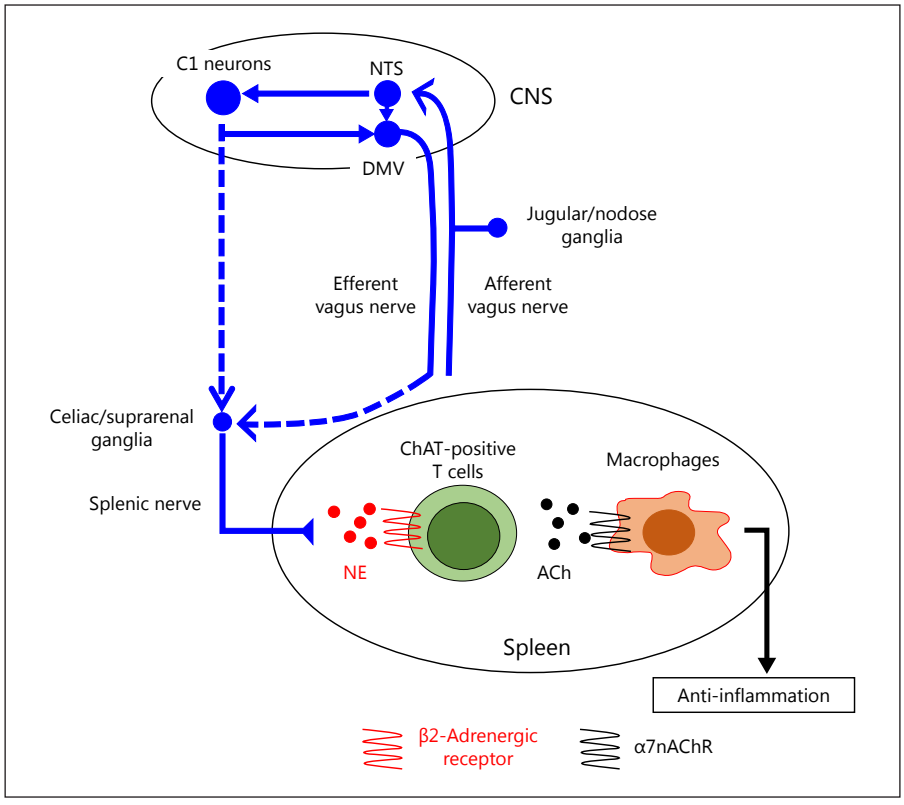

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Fig. 1. The inflammatory reflex. The inflammatory reflex originally consisted of the afferent/efferent vagus nerve, splenic nerve, choline acetyltransferase (ChAT)-positive T cells expressing β2-adrenergic receptors, and macrophages expressing α7 nicotinic acetylcholine receptors (α7nAChRs). Recent studies have shown that the reflex is much more complicated than what it was before (e.g., a sympathetic route in the stimulation of C1 neurons). ACh, acetylcholine; CNS, central nervous system; DMV, dorsal motor nucleus of the vagus; NE, norepinephrine; NTS, nucleus tractus solitarius.

Restrain Stress and Optogenetic Stimulation of C1 Neurons: Is Stress Good for the Kidney?

Optogenetics is an emerging technique that regulates the activity of neurons. It allows for targeted excitation or inhibition with cellular specificity that is not feasible with electrical stimulation. In optogenetic stimulation, light-reactive membrane proteins (e.g., channelrhodopsin) are expressed specifically in some neurons in transgenic mice or by injecting cre-dependent viral vectors. When a specific wavelength light (blue light in the case of channelrhodopsin) is applied to the target nerve in these mice, the opsins react to the light, resulting in selective activation or silencing of the target neurons [17].

By using this technique, our recent work has added some new aspects to the inflammatory reflex [18]. C1 neurons, residing in the medulla oblongata and innervating sympathetic efferents, dorsal motor nucleus of the vagus nerve, and paraventricular nucleus of the hypothalamus, mediate adaptive autonomic responses to various stressors (e.g., hypoxia, hypotension, and lipopolysaccharide) [19]. Optogenetic stimulation of C1 neurons protected mice from renal IRI, and the protective effect was dependent on the spleen, β2-adrenergic receptors, and α7nAChRs, which indicates that the CAP is involved in the protective effect of C1 neuron stimulation. It is interesting that restraint stress for 10 min also protected the kidney from IRI, which was mediated by C1 neurons. Given that various physical stresses including restraint stress activate C1 neurons, these results indicate that physical stresses should be avoided as much as possible in studies of AKI because these stresses potentially cause a confounding effect. The protection was significantly decreased by ganglionic blockade but unaffected by subdiaphragmatic vagotomy or by corticosterone receptor blockade, suggesting that C1 neurons activate the CAP through a sympathetic, not a vagus, route (Fig. 1).

Pulsed Ultrasound

Pulsed ultrasound (US) is one of the promising non-pharmacological and noninvasive methods to prevent AKI. We showed that prior US application can protect kidneys from IRI in mice using a clinical US machine [20, 21]. US suppresses systemic inflammation and attenuates AKI probably through activating the CAP. This is supported by various experiments including splenectomy, splenocyte transfer, CD4 reconstitution in *Rag1*-deficient mice, which lack T and B lymphocytes, and α7nAChKO mice experiments. Bone marrow chimera experiments

further revealed that α7nAChRs on hematopoietic, but not parenchymal cells, are responsible for mediating the protective effect of US [21]. The protective effect of US was also confirmed in a cecal ligation and puncture-induced sepsis model [21].

Other Strategies

Remote ischemic preconditioning (RIPC) is a technique for producing resistance to a subsequent sustained episode of ischemia after a brief episode of ischemia being applied in distant tissues or organs. In addition to animal studies, many clinical studies on human AKI have been done [22, 23]. They mainly use a blood pressure cuff to briefly restrict blood flow to the lower limb or upper limb (total ischemic duration is 15–30 min) [22]. The latest systematic review and meta-analysis show that RIPC significantly decreased the incidence of AKI from 23.3 to 11.5%. They also found that RIPC significantly reduced the incidence of AKI in the contrast-induced AKI subgroup but not in the ischemia reperfusion-induced AKI subgroup.

Another interesting nonpharmacological approach to achieving prevention of inflammation is acupuncture. Recently, Torres-Rosas et al. [24] showed that sciatic nerve activation with electroacupuncture suppresses systemic inflammation and rescues mice from polymicrobial peritonitis induced by cecal ligation and puncture by activating the vagus nerve. They identified a new sciatic-to-vagus neural circuit that does not require α7nAChRs or β2-adrenergic receptors, suggesting that the new circuit is different from the classical CAP. This new anti-inflammatory mechanism mediated by the sciatic and vagus nerves induces the production of dopamine from the adrenal glands and dopamine release contributes to the suppression of systemic inflammation. The activation of this new circuit has not been applied to other organs, but the effect seems promising for the kidneys.

Conclusion

Activating the CAP is an emerging therapeutic strategy against inflammatory diseases, including AKI. Several methods to activate the CAP, such as VNS and pulsed US, have successfully reduced inflammation in animal models. These strategies appear promising as a therapy for AKI, although its effectiveness and safety in humans should be confirmed in future clinical trials. Novel techniques (e.g., optogenetics) should help us to elucidate the mechanisms by which VNS and pulsed US activate the CAP and ameliorate the inflammation.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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