Neuroimmune Control of Acute Kidney Injury and Inflammation

Tsuyoshi Inoue · Mark D. Okusa

Department of Medicine, Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, University of Virginia Health System, Charlottesville, Va., USA

Key Words

Neuroimmunomodulation · Immunity · Inflammation · Acute renal failure · Vagus nerve · Kidney · Autonomic nervous system

Abstract

Despite major advances in identifying pathophysiological mechanisms of acute kidney injury (AKI), no definitive therapeutic or preventive modalities have been developed with the exception of dialysis. One possible approach is the control of inflammation and AKI through activation of the neuroimmune axis. The cholinergic anti-inflammatory pathway is thought to contribute to the homeostatic response in inflammation-related disorders and forms the basis for recent approaches toward therapeutic intervention. The concept is based on the emerging understanding of the interface between the nervous and immune systems. In the cholinergic anti-inflammatory pathway, the efferent vagus nerve indirectly stimulates the CD4⁺ T-cells in the spleen. The CD4⁺ T-cells produce acetylcholine, which stimulates $\alpha 7$ nAChRs on macrophages. Activation of the $\alpha 7$ nAChRs on macrophages in turn activates NF- κ B and elicits an anti-inflammatory response. Recently, we demonstrated the effect of a non-pharmacologic, noninvasive, ultrasound-based method to prevent renal ischemia-reperfusion injury and sepsis-induced AKI in mice. Our data suggest that ultrasound-induced tissue protection is mediated through the activation of the cholinergic anti-inflammatory pathway. In addition, nicotinic receptor agonists and ghre-

lin, a neuropeptide, were reported to prevent AKI possibly through a mechanism closely linked with the stimulation of the vagus nerve. Based on the studies focusing on inflammation and the observations regarding kidney injury, we believe that activating the cholinergic anti-inflammatory pathway will be a new modality for the prevention and treatment of AKI.

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Introduction

Acute kidney injury (AKI) is a common and major concern in hospitalized patients because of its high morbidity and mortality [1]. Current therapies for AKI are mainly supportive, and there are no definitive therapeutic or protective modalities available for AKI despite research that has identified many potential pathogenic mechanisms, such as inflammation and local and systemic factors. Among them, neural control of inflammation is emerging as an important concept, yet very little is known regarding how neural factors can modulate inflammation that develops after AKI.

The nervous and immune systems have been thought to serve separate and distinct functions; however, recent-

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ly there has been considerable evidence that indicate these 2 systems are linked to maintain normal homeostasis as well to respond to stress and pathophysiological conditions that are characteristic of certain disorders [2]. Immune cells express receptors for neurotransmitters that permit control of immune response against infection by the central and peripheral nervous systems. Some immune cells can synthesize and secrete neurotransmitters, such as acetylcholine (ACh)-secreting lymphocytes. One well-known mechanism of neuroimmune control of inflammation involves the adrenal glands, that is, the hypothalamic–pituitary–adrenal axis [3]. In response to stress messages from the brain, the adrenal glands release hormones into the blood, and these affect almost all types of immune cells. The immune cells perform immunosuppressive and anti-inflammatory functions through various genomic and non-genomic mechanisms [3]. Neuropeptides, a type of neuromodulator released from neurons, have both pro- and anti-inflammatory effects [4]. In addition, recent advances, demonstrating a further link between the nervous and immune systems, revealed that vagus nerve stimulation (VNS) regulates the immune system [5] and serves as a target for neuroimmunomodulation of peripheral nerve activity in disorders such as myocardial infarction, colitis, pancreatitis, ischemia-reperfusion injury, sepsis, arthritis and others [6].

VNS, as a means of modulating peripheral nerve activity, suppresses inflammation, and clinical trials examining the effects of VNS on inflammatory disorders, such as rheumatoid arthritis [7] and inflammatory bowel diseases are ongoing [8]. Recent studies show that activation of both pro- and anti-inflammatory immune responses occurs promptly after sepsis [9]. The balance of these opposing responses may lead to a favorable outcome. A hyper-proinflammatory response may lead to early death; however, it is thought that this response is controlled in part by an efferent arm of the inflammatory reflex, referred to as the cholinergic anti-inflammatory pathway, which involves norepinephrine and ACh, neurotransmitters of the sympathetic and parasympathetic nervous system, respectively. In this review, we highlight the neuroimmune axis in AKI and the possibility of neural modulation of this pathway for future treatment.

Cholinergic Anti-Inflammatory Pathway

Recent studies support the concept that a potent anti-inflammatory mechanism is mediated by the cholinergic anti-inflammatory pathway [10]. Stimulation of

efferent vagus nerve activity is known to cause decreased heart rate, induction of gastric motility, dilation of arterioles and constriction of pupils. In addition, VNS has also been demonstrated to inhibit the inflammatory response [10]. In this study, electrical stimulation was performed 30 min before systemic administration of lipopolysaccharide (LPS 15 mg/kg, intravenously), which is used to mimic sepsis in rats. Blood was collected from the right carotid artery 1 h after LPS administration, and serum tumor necrosis factor alpha (TNF α) concentrations were quantified. Activation of the cholinergic anti-inflammatory pathway by direct electrical stimulation of the efferent vagus nerve inhibits the synthesis of TNF α , a cytokine involved in systemic inflammation that is mainly produced by macrophages. Vagotomy significantly exacerbates TNF α responses to inflammatory stimuli and increases the mortality of animals injected with endotoxin (LPS). A key role of the $\alpha 7$ subunit of the nicotinic acetylcholine receptor ($\alpha 7$ nAChR) in controlling inflammation was identified using $\alpha 7$ nAChR-deficient mice [11]. Electrical stimulation of the vagus nerve inhibited TNF α synthesis in wild-type mice, but failed to inhibit TNF α synthesis in $\alpha 7$ nAChR-deficient mice. Furthermore, macrophages derived from $\alpha 7$ nAChR-deficient mice were refractory to cholinergic agonists, whereas those from wild-type mice produce TNF α in the presence of nicotine or ACh [11].

In this reflex pathway, afferent signals to the brain are initiated by damage-associated molecular pattern molecules, including endogenous products released by dying cells, and/or pathogen-associated molecular patterns from bacterial pathogens that activate toll-like receptors and invoke afferent signals from the vagus nerve to the brain. The brain sends efferent signals to inhibit cytokine production via pathways dependent on $\alpha 7$ nAChR expressed on macrophages and other cells [6].

Immune Cells in Spleen Play Important Roles in VNS

The spleen plays an important role in the immune system and was found to be essential for the inhibition of systemic inflammation by VNS [12]. To explore the relationship between the anti-inflammatory pathway and organs, organ TNF α concentrations during lethal endotoxemia in rats were measured [12]. The spleen was revealed to be an important source of TNF α production that is regulated by VNS. VNS stimulation of TNF α production is attenuated in splenectomized animals. More-

Fig. 1. Cholinergic anti-inflammatory pathway. The cholinergic anti-inflammatory pathway links the nervous and immune systems. Activation of the pathway by VNS suppresses inflammation, and enhancing this pathway protects kidneys from acute injury. The efferent vagus nerve stimulates CD4-positive T-cells in spleen via the splenic sympathetic nerve. Release of norepinephrine binds to beta adrenergic receptors on CD4+ T-cells, which then elicits release of ACh. ACh binding to $\alpha 7$ nAChRs on macrophages produces an anti-inflammatory response, such as TNF α suppression. Ultrasound prior to kidney ischemia and reperfusion attenuates injury through either a neuronal pathway or neuronal independent pathway that requires the spleen, CD4+ T-cells and $\alpha 7$ nAChRs expressed on bone marrow-derived cells [17, 18]. US = Ultrasound; NE = norepinephrine; M Φ = macrophage; ACh = acetylcholine; TNF α = tumor necrosis factor alpha.

over, VNS in $\alpha 7$ nAChR-deficient mice failed to inhibit TNF α production in the spleen. Considering these results, $\alpha 7$ nAChR-positive cells in spleen appear to be important for the anti-inflammatory effect produced by VNS.

Parasympathetic neurons, such as the vagus nerve, release ACh from their nerve terminals. Although the spleen contains ACh, the vagus nerve does not appear to innervate the spleen [13]. Nerve fibers in spleen, originating in the celiac ganglion, are adrenergic, not cholinergic, and produce norepinephrine as the primary neurotransmitter. The source of splenic ACh was unexplained following inflammatory reflex stimulation. Rosas-Ballina et al. [14] found that CD4-positive T-cells released ACh in response to stimulation by norepinephrine. Within minutes after electrical VNS, ACh levels were elevated in the spleen and reached peak levels within 20 min. To identify the cells that produce ACh, choline acetyltransferase (ChAT)-enhanced green fluorescent protein mice, which express enhanced green fluorescent protein under the control of transcriptional regulatory elements for ChAT, the enzyme that catalyzes the biosynthesis of ACh, were used. Flow cytometry revealed that CD4-positive T cells expressing ChAT can be defined phenotypically as CD44^{high} CD62L^{low} memory T-cells.

Thus ACh-synthesizing CD4+ T-cells appear to be an important link between the vagus nerve and the anti-in-

flammatory role of the spleen [14]. ACh, released from these cells, binds to $\alpha 7$ nAChR on macrophages and suppresses TNF α release (fig. 1). Although many of the functional components of the cholinergic anti-inflammatory pathway have been defined, including the activation of CD4+ T-cells in spleen in response to efferent VNS, the precise mechanisms are yet to be identified.

Neuroimmune Control of Inflammation in AKI

Although a direct effect of VNS in modulating AKI has not yet been reported, there is one paper that supports the role of the cholinergic anti-inflammatory pathway in AKI [15]. They examined the effects of cholinergic stimulation using agonists, nicotine (1 mg/kg) or GTS-21 (10 mg/kg), in renal ischemia-reperfusion injury in rats [15]. Prior administration of either of the agonists (20 min before clamping the renal vessel) significantly attenuated renal dysfunction and tubular necrosis induced by renal ischemia. GTS-21 administered 2 h after reperfusion did not significantly improve renal function. In addition, they showed that TNF α protein expression and leukocyte infiltration of the kidney were markedly reduced by prior cholinergic agonist treatment.

Ghrelin, a peptide produced by cells in the gastrointestinal tract, which also functions as a neuromodulator,

attenuates AKI [16, 17]. In the ischemia-reperfusion injury model of AKI in rats (60 min ischemia and 24 h reperfusion), kidney injury was attenuated by human ghrelin (4 nmol/rat) when slowly infused over 30 min soon after reperfusion; the protective effect was abolished by prior vagotomy [16]. Ghrelin (100 µg/kg/i.p.) had similar protective effects in a cecal ligation puncture-induced sepsis model of AKI in rats [17]. These studies demonstrate that ghrelin, a neuropeptide regulated by the vagus nerve, may have potent effects to attenuate AKI.

Ultrasound Treatment Modulates the Cholinergic Anti-Inflammatory Pathway and Attenuates AKI

Recently, we showed that prior ultrasound application can protect kidneys from ischemia-reperfusion injury in mice [18]. In these studies, we used a clinical Sequoia 512 ultrasound machine with a 15L8w transducer (Acuson, Malvern, PA). The ultrasound treatment consisted of ultrasound pulses administered with a bursting mechanical index of 1.2 at a frequency of 7 MHz. Ultrasound pulses were 1 s in duration and were applied once every 6 s for 2 min. The protective effect of ultrasound was lost in *Rag1*-deficient mice, which lack T- and B-lymphocytes. Adoptive transfer of wild-type CD4⁺ T-cells into these mice prior to ultrasound rescued the tissue protective effect, thereby demonstrating the requirement for CD4⁺ T-cells. In addition, when the spleen was removed before CD4⁺ T-cell transfer, this protection again was abolished [18]. Splenic sympathectomy with splenic injections of 6-hydroxydopamine, a neurotoxin that selectively destroys catecholaminergic nerve terminals, exacerbates renal ischemic-reperfusion injury [19]. These results, taken together, reveal that the spleen, splenic sympathetic nerve and CD4⁺ T-cells mediate the protective effects of ultrasound. In addition, loss of α7nACh response, either by pharmacologic antagonism or genetic deficiency of the α7nAChR on bone marrow-derived cells, blocked the protective effect of ultrasound [18]. Ultrasound protection was associated with reduced expression of circulating and kidney-derived cytokines such as TNFα. Also the protective effect of ultrasound was confirmed in a cecal ligation puncture-induced sepsis model [19] as well as in a pigs subjected to ischemic-reperfusion injury (unpublished observations, J. Gigliotti and M.D. Okusa, 2015). These results support the concept that prior ultrasound prevents AKI through the cholinergic anti-inflammatory pathway.

Clinical Use of Vagus Nerve Stimulator

VNS therapy, by using a device that is surgically implanted to stimulate the vagus nerve, was approved for the treatment of medically refractory epilepsy in Europe in 1994 and in the United States in 1997 by the US Food and Drug Administration. The Food and Drug Administration approved VNS therapy for treatment-resistant depression in 2005. As of August 2014, over 100,000 VNS devices were implanted in more than 75,000 patients worldwide [20] and clinical trials on a wide variety of disorders, such as heart failure, hypertension, inflammation and diabetes, are on-going. The pulse generator is implanted under the left clavicle, and the stimulation lead is wrapped around the left vagus nerve in the neck [21]. Postoperative infections occur in approximately 3% of patients but can be treated with oral antibiotics. Side effects are related to the stimulation. Cough, hoarseness, voice alteration and paresthesias are commonly observed, and these side effects tend to diminish with time.

Recently, 2 non-invasive external devices have been developed to stimulate the vagus nerve through the skin [22]. One can provide transcutaneous VNS by using a dedicated intra-auricular electrode (like an earphone) that stimulates the auricular branch of the vagus nerve. Another can deliver a proprietary, low-voltage electrical signal to the cervical vagus nerve. The effect of these 2 devices is currently being investigated.

Conclusion

The neuroimmune pathway is a critical interface that permits rapid modulation of the immune system through a reflex pathway referred to as the cholinergic anti-inflammatory pathway. This pathway provides an exciting new target with the potential for modulating a number of inflammatory conditions, including myocardial infarction, colitis, rheumatoid arthritis, AKI and others. In most cases to date, invasive procedures are necessary. Direct VNS or pharmacological agents have been used but may be limited by the invasiveness of the procedure to insert devices and toxicity associated with pharmacological agents. Our studies demonstrate that pulses of ultrasound, a non-invasive, non-pharmacological tool, can activate the cholinergic anti-inflammatory pathway and thereby largely attenuate AKI. Additional studies will lead to further understanding of neuroimmunomodula-

tory mechanisms of inflammatory disorders and to improved neuroimmunomodulatory therapies to preserve organ function.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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