Aim of review: The hyper-inflammatory response is the classical pathogenesis of sepsis course. It has been shown that efferent vagal nerve stimulation (VNS) can regulate inflammatory response through a combination of acetylcholine and special α7 nico
tinic acetylcholine receptor expressed on macrophages, which is defined as the cholinergic anti-inflammatory pathway (CAP). Furthermore, therapeutic methods related to CAP have been studied on lots of pathologic conditions, such as sepsis, ischemia/reperfusion injury, pancreatitis and hemorrhagic shock, etc. The aim of this review is to provide the evidence that VNS may be an effective adjuvant treatment bringing benefits for prognosis of sepsis by controlling excessive inflammatory response.

Methods: We searched literatures published in the Pubmed with keywords "inflammation", "sepsis" and "vagal nerve stimulation (VNS)" from January, 1986 to November, 2016, analyzed them and then assessed the evidence as to whether VNS may be an effective adjuvant for treatment of sepsis. In this review, the evidence regarding the role of hyper-inflammatory response in the pathogenesis of sepsis and inflammatory regulation of CAP will first be described. Then, the possible beneficial effects of VNS on inflammatory response of sepsis by modulating CAP will be provided.

Recent findings: Sepsis is complex interactions between infecting microorganism and host's immune, inflammatory, and coagulation systems. The beneficial effects provided by CAP activation have been demonstrated on lots of pathologic conditions related to inflammation. Furthermore, VNS has been shown to promote the gastrointestinal motility and provide protection of intestinal barrier, reducing organ injury. In addition, VNS has been effectively used for treatment of some diseases in clinical practice.

Summary: There is limited number of effective therapeutics available for septic patients. Because the CAP plays an important role in the regulation of inflammatory response, we consider that with the standard intensive care therapy, VNS may be an effective adjuvant treatment bringing benefits for prognosis of sepsis by controlling excessive inflammatory response. If efficiency of this new intervention is proved by clinical experiments, it may represent an exciting opportunity to develop novel therapeutics recovering unregulated inflammatory response in septic patients.
Sepsis is defined as life-threatening organ dysfunction caused by disordered reaction to an infection (1) and is a major public health concern, costing more than $20 billion (5.2% of total US hospital costs) in 2011 (2). Despite many efforts have been performed, the reported incidence of sepsis is still increasing (3). Furthermore, sepsis-related cognitive impairment and functional disability have become a serious burden for our society and these sequels have spoiled life equality of survivors seriously (4). Up to now, thus, a huge challenge that the clinical physicians must face still is to develop feasible and effective treatments for sepsis.

Hyper-inflammatory response has been considered a significant role in the pathogenesis of sepsis (5). It is generally believed that the communication among the immune, nervous and endocrine system is essential for the regulation of inflammatory response. Innate immune responses are critical for protection against lethal infection and tissue injury, but the uncontrolled production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), and high mobility group box-1 (HMGB1), cause the development of severe sepsis (5, 6). In most septic patients, the presence of early signs also supports that the disease is resulted from an overwhelming inflammatory response to the underlying infection (7). The nervous system, through the vagal nerve, can modulate inflammation by decreasing the release of pro-inflammatory cytokines from macrophages. This anti-inflammatory effect is mediated by an interaction of acetylcholine with α7 subunit-containing nicotinic acetylcholine receptor (α7nAChR) on the macrophages and other immune cells. It is known as the cholinergic anti-inflammatory pathway (CAP) (8).

With recognition of the CAP, more attention has been paid to it with expectation of controlling inflammatory integrally. It has been shown that acetylcholine, an important vagal neurotransmitter, can significantly attenuate the release of pro-inflammatory cytokines, i.e., TNF-α, IL-1β and IL-6, but not the anti-inflammatory cytokines, such as IL-10 (8). In the lethal endotoxemic rats, moreover, direct electrical stimulation of the peripheral vagal nerve can inhibit TNF-α synthesis in liver, decrease peak level of plasma TNF-α, and prevent the development of shock (9). This suggests that the CAP plays an important role in the regulation of inflammatory response. In this review, we describe the evidence regarding the role of hyper-inflammatory response in the pathogenesis of sepsis and inflammatory regulation of CAP, and then provide an overview regarding the possible beneficial effects of vagal nerve stimulation (VNS) on inflammatory response of sepsis by modulating CAP.

### Hyper-Inflammatory Response Participates in the Pathogenesis of Sepsis

Sepsis is complex interactions between infecting microorganism and host’s immune, inflammatory, and coagulation systems (10). Also, the pathogenesis of sepsis is generally considered to be involving hyper-inflammatory response that produces cytokines excessively and activates the immune system aberrantly (11, 12). Available literatures demonstrate that loss of balance between inflammatory and anti-inflammatory responses occurs during sepsis, leading to reprogramming of immune cell activities and consequently bringing about irreversible tissue injury and multi-organ failure.

Different inflammatory cytokines control distinct stages of inflammation response in sepsis. At the initial phase, bacterial products bind to Toll-like receptors (TLR) on macrophages (13), which stimulates intracellular signaling and increases the transcription of pro-inflammatory cytokines, such as TNF-α and IL-1β. At the same time, anti-inflammatory cytokines such as IL-10 are produced. Subsequently, TNF-α provokes the neutrophil-mediated tissue injury by acting on the endothelial cells and neutrophils, and increases the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (14). Also, it causes the endothelial dysfunction through activation of xanthine oxidase and production of superoxide (15). Neutrophils are large and stiff cells, and their adhesion with capillary endothelial cells may affect microcirculation of organs (16). Meanwhile, activated neutrophils can cause the endothelial cell damage, which promotes vascular permeability and the capillary leak syndrome (17). Moreover, TNF-α can activate neutrophils...
by up-regulating the integrin adhesiveness and promoting the extravasation to the lung, liver and gut. It has been demonstrated that extravasated neutrophils can damage tissues by releasing oxygen free radicals and proteases. Also, TNF-α can amplify the inflammatory cascade by the autocrine and paracrine manners, because activated macrophages or monocytes secrete other pro-inflammatory cytokines (11).

During the late phrase of sepsis, HMGB1 begins to become the dominated character of mediating inflammation (18). HMGB1 is an indispensable pro-inflammatory cytokine and can induce TNF-α release significantly later than lipopolysaccharide (LPS) does. Moreover, this induction takes place with two peaks. The first is at approximately 3 hours after HMGB1 exposure, and the second is at 8-10 hours after that. TNF-α and HMGB1 can promote macrophages, endothelium and neutrophils to produce the pro-inflammatory cytokines. Besides as a later mediator during the systemic inflammatory response syndrome (SIRS), HMGB1 possesses many organ special biological functions, including increasing bacteria translocation and mucosa permeability in gut, and inducing arrhythmia (19). Thus, HMGB1 is a necessary mediator of organ damage in severe sepsis. Even, macrophages have also been shown to release HMGB1 when they are exposed to both apoptotic T cells and apoptotic macrophages (20). This phenomenon is contrast to the general idea that the apoptotic cells are cleared via a non-inflammatory pathway. In fact, the accumulation of apoptotic cells may provoke the release of HMGB1, which, in turn, mediates severe organ damage.

In view of the critical role of inflammation in pathogenesis of sepsis, several studies have been carried out to find effective measures to ameliorate the disease course by halting inflammatory cascades (21-25). Applying antibody of TNF-α has been tested to relieve inflammation during sepsis in pre-clinical studies, though the successful application in patients still need more studies to verify (26). Grégoire et al. (21) found that HMGB1 accumulation could exert influence on neutrophil-dependent antibacterial defense mechanisms, and blocking HMGB1 by anti-HMGB1 Ab could mitigate neutrophil NADPH oxidase activity caused by sepsis-induced dysfunction. Additionally, patients recovering from septic shock even got a preservation of neutrophils ability, with more competence to activate NADPH oxidase and clear bacterium. Inhibiting inflammatory response by Butyrate could improve sepsis-related heart dysfunction in mice (22), as demonstrated by attenuation of septic heart depression and reduction of morphological changes of myocardium. The pro-inflammatory factors, TNF-α, IL-6 and LTB4 decreased significantly compared with placebo group. Regulating hyper-inflammatory response in sepsis animals by Annexin A5 could affect the interaction between HMGB1 and TLR4 and then obtain a survival amelioration (27). Even when the treatment was given after the early cytokine response, it still could protect against endotoxin lethality, which further indicated the important role of HMGB1 during the later phrase of sepsis. Repressing TLR-triggered inflammation response in sepsis could provide protection against lung injury (28). Based on the above-mentioned, it may be conferred that suppressing inflammation can provide a protection for sepsis. However, the above approaches are all focused on one kind or a few kinds of pro-inflammatory cytokines with limited effect, and this may partially explain why some clinical studies using method aimed at special cytokine do not show a significant protection against the development of sepsis (26). Thus, there is an urgent need to explore some measures which can modulate hyper-inflammation integrally and powerfully, otherwise just targeting one or several cytokines.

Both afferent and efferent vagal nerves have been shown to be involved in the regulation of inflammatory response (Figure 1). Efferent vagus nerve originates from the dorsal motor nucleus (DMN) and carry outbound signals to reticuloendothelial system. The nervous system Activate efferent vagus nerve and then increases the release of acetylcholine, which, in turn, binds on α7nAChR on the macrophages and other immune cells, rapidly inhibiting the synthesis or release of pro-inflammatory cytokines (29).
Moreover, the effects of some anti-inflammatory agents, such as galantamine, cholecystokinin (CCK) and semapimod, are mediated by vagus nerve (30-32). CAP is a direct and rapid endogenous mechanism with relatively quicker response than the humoral anti-inflammatory pathways (9). It plays a crucial role for fine tuning of inflammation integrally and stimulating any part of the pathways that can directly inhibit production and release of pro-inflammatory cytokines (29).

CAP can modulate the hyper-inflammatory response during sepsis. The current data has shown that activating CAP by dealing with any portion of the pathway can provide the protective effect against sepsis. It has been shown that over-excitation of the sympathetic system can result in sympathetic-vagal imbalance in septic animals (33). Stimulating efferent vagus nerve can be used to rebalance the sympathetic and vagal systems, which may be meaningful for septic patients. In sepsis mice, stimulation of the CAP by nicotine can mitigate inflammatory cytokines, attenuate organ failure and improve sepsis-induced mortality, which is related with the inhibition of TLR4 expression via α7nAChR/PI3K signaling pathway (34). As we all know, the TLR4 plays an important role in the induction of the hyper-inflammatory response and tissue injury in sepsis. This indicates that stimulating CAP can suppress inflammation overall and produce extra benefits beyond it. Li et al. (35) applied α7nAChR agonist to activate CAP in sepsis mice and showed a powerful anti-inflammatory effect with excessive reduction of TNF-α. Targeting the terminal receptors of CAP- α7nAChR may confer a novel therapeutic modality to treat hyper-inflammatory disease states. Importantly, a
significant anti-inflammatory effect of α7nAChR agonist has been reproduced in human experiments (36), and the essential role of α7nAChRs for TNF-α production has been confirmed (37). Furthermore, VNS has been shown to suppress the production of TNF-α and other early pro-inflammatory cytokines, and decrease the release of HMGB1.

Up to now, several studies focused on activation CAP by VNS have obtained promising results. In endotoxin-induced intestinal tight junction injury mice, Zhou et al. (38) found that VNS can ameliorate the damage of tight junction ultrastructure by suppressing translocation of nuclear factor κB p65 and downregulating myosin light chain kinase. Furthermore, α7nAchR may play a crucial role in this process. It is the first time to confirm that VNS mitigates the disruption of tight junction in intestinal epithelium in endotoxemic mice. In addition, VNS can significantly decrease concentrations of the pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, alleviating LPS-induced acute lung injury (39). Li et al. (40) find that VNS can ameliorate general and local inflammation in LPS-related sepsis, improve brain function and suppress the development of sepsis-related brain diseases. On the other hand, loss of vagal tone can aggravate systemic inflammation in endotoxemic rats and even impair cardiac function, as demonstrated by elevated cytokine levels in plasma and ventricular tissue (41). Actually, during initial LPS infusion, even a brief VNS is enough to reverse these inflammatory effects.

All these results indicate that CAP is indeed involved in the regulation of inflammatory responses at both early and late stages of sepsis, and plays an important role in maintaining normal organs function. Modulating CAP can confer a powerful protection by acting on lots of pro-inflammatory cytokines overall, and improve sepsis-related organ injury. Thus, activating CAP by some practice, such as VNS, maybe a useful adjuvant for sepsis (Figure 2).

### Feasibility of Using VNS as an Adjunct to Treatment of Sepsis

VNS is an effective method to activate CAP and this measurement has been studied in several animal sepsis models. In the sepsis rat model, Huston et al. (42) performed the transcutaneous VNS by direct pressing the border of the trachea and improved animal survival. Several animal experiments have demonstrated the treatment effect of VNS on sepsis as above mentioned and exhibited promising results. Based on these findings, it can be concluded that VNS displays a significant inflammation amelioration in sepsis animals. Therefore, we speculate that VNS may be a valuable treatment for alleviating hyper-inflammatory response of sepsis. In addition, breakdown of intest-
tinal barrier exacerbates the condition of sepsis (43). The VNS has been shown to reduce intestinal inflammation (44), provide protection of intestinal barrier, and reduce organ injury (45). This means that VNS can provide benefits beyond modulation of inflammation.

Beside the successful application in animal models, VNS has evolved versatile patterns for practice and been used in both preclinical and clinical studies. The direct VNS has been proved as an effective treatment for seizure disorder and major depression. These clinically approved devices can chronically activate afferent vagal signaling that affects brain function (46). Because direct VNS requires an invasive procedure associated with the side-effects and a relatively high financial cost, a non-invasive method of VNS would be a more suitable way for the treatment of severe sepsis in clinical practice. The transcutaneous VNS can be achieved by placing electrode in the external auditory canal at the inner side of the tragus. In this way, stimulation is conducted via the auricular nerve to the jugular ganglion, from there through the vagal nerve into the medulla oblongata and to the nucleus of tractus solitarius (NTS) (47). NTS is a major site for termination of vagal afferents, and NTS neurons then project to DMN where most of the efferent vagal preganglionic fibers originate (8). In cases of refractory epilepsy, epilepsy, depression, heart disease, angina pectoris and hypertension, heart failure and cardiac arrhythmias (48), VNS has been shown a promising effect and these successful applications on patients have indicated that implantable VNS appear to be safe and well tolerated. These evidences further suggest that the VNS may have a beneficial effect on inflammatory response of sepsis by modulating the CAP, and could be a feasible measurement.

Prospects

It has been clear that further development of an effective treatment scheme of sepsis is still be required. This should be a combination of the different therapy ways because the evil needs to be attacked with multiple weapons and there is not one magic bullet to control it. Given the pronounced anti-inflammation effect of VNS in sepsis animals, combining the safe utilization for epilepsy, depression, heart disease, angina pectoris and hypertension in pre-clinical and clinical studies, we speculate that VNS may be useful to alleviate the hyper-inflammation of sepsis patients.

As compared to the direct VNS, several advantages of the transcutaneous VNS exist. First, the procedure is simple and feasible in clinical practice. Second, it does not significantly increase the expenditure. Third, it may avoid the infection, one of the most common side effects of the VNS, and other complications of the VNS (49) because of its non-invasive feature. Fourth, the transcutaneous VNS has the same effect as direct VNS (47). Fifth, theoretically, it may not increase the risk of death to critical ill patients. Therefore, the transcutaneous VNS performed on critical ill patients is ethically permissible.

In the available literature, however, there is no coincident results regarding the exact role of VNS in human sepsis. A randomized controlled study on experimental human endotoxemia demonstrates that transvenous VNS can not regulate innate immune response and the inflammatory responses in the VNS group are similar to these in the control (50). Perhaps, it is possible that the trial examples included are healthy volunteers with LPS administration, which can not imitate complicated severe sepsis in patients and therefore is difficult to get positive results. On the other hand, Cedillo et al. (51) carried out a pilot study to search the regulatory pathway in human sepsis, and found that α7 gene expression levels were closely associated with inflammation control and the prognosis, indicating that CAP does exist in sepsis patients and its effluent pathway receptor may be a useful biomarker for assessing prognosis for sepsis patients.

We believe that if efficiency of this new intervention is proved by clinical experiment, it may represent an exciting opportunity to develop novel therapeutics recovering unregulated inflammatory response in septic patients. Although the VNS is unlikely replace the standard intensive care therapy, we hope that it becomes an adjunct to benefit septic patients.

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