Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review Article

The role of afferent pulmonary innervation in ARDS associated with COVID-19 and potential use of resiniferatoxin to improve prognosis: A review

Alexis Nahama *, Roshni Ramachandran, Alvaro Francisco Cisternas, Henry Ji

Sorrento Therapeutics Inc., 4955 Directors' Place, San Diego, CA, 92121

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ABSTRACT

Acute respiratory distress syndrome (ARDS) is one of the major causes of mortality associated with COVID-19 disease. Many patients will require intensive care with ventilatory support. Despite progress and best efforts, the mortality rates projected remain high. Historical data outlook points towards 80% expected fatality for patients progressing to advanced pulmonary disease, even when hospitalized in the intensive care unit. This is particularly true among the patient population over 65. Novel life-saving strategies are desperately needed to mitigate the high mortality that will be associated with the late stage SARS-CoV-2 viral infection associated with the fatal respiratory distress.

We hypothesize that the morbidity, severity of the disease, and underlying physiological events leading to mortality are closely linked to the TRPV1 expressing neuronal system (afferent/efferent neurons) in the lungs. TRPV1 expressing cells are responsible for pain transmission, inflammation and immunomodulation throughout the entire pulmonary system and are modulating the processes associated with localized cytokine release (storm) and overall rapid disease progression.

We suggest that therapeutic approaches targeting TRPV1 containing nerve fibers in the lungs will modulate the inflammatory and immune signal activity, leading to reduced mortality and better overall outcomes. We also propose to further explore the use of resiniferatoxin (RTX), an ultra-potent TRPV1 agonist currently in clinical trials for cancer and osteoarthritis pain, as a possible ablating agent of TRPV1 positive pulmonary pathways in patients with advanced COVID-19 disease.

Contents

Respiratory failure due to acute respiratory distress syndrome (ARDS) is one of the major causes of mortality (53%) associated with COVID-19 disease [1]. Around 10 % of the patients require intensive care unit (ICU) care with ventilatory support and an ICU mortality rate of 79% has been reported [2,3].

The severity of the disease is higher in older patients with 80 % death observed in those over 60-65 years of age [1,4], while younger infected patients seem to be less susceptible and exhibit medium-mild symptoms [23].

Once the lower respiratory tract is affected, the respiratory distress progresses very quickly, with time to death reported as rapidly as 14 days from initial symptoms despite availability of ventilator palliative support [1]. It has been proposed that the severity and mortality rates of the susceptible population infected by COVID-19 is related to a cytokine storm, in which an exaggerated production of pro-inflammatory substances are released into the pulmonary microenvironment over a short period of time [5].

Novel life-saving strategies are desperately needed to mitigate the high mortality that is associated with the late stage viral infection with acute respiratory distress.

* Corresponding author.
E-mail address: Anahama@sorrentotherapeutics.com. (A. Nahama).

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We hypothesize that the morbidity, severity of the disease, and underlying physiological events linked to mortality can be explained by the involvement of the TRPV1 expressing neuronal system (afferent/efferent neurons). TRPV1 positive pathways are responsible for pain transmission, inflammation and immunomodulation throughout the entire pulmonary system. We suggest that therapeutic approaches targeting TRPV1 expressing neurons in the lungs will modulate the inflammatory and immune signal activity, leading to reduced mortality and better overall outcomes. We propose to further explore the use of resiniferatoxin (RTX), an ultrapotent TRPV1 agonist currently in clinical trials for cancer and osteoarthritis pain, as a possible ablating agent of TRPV1 positive pulmonary pathways in patients with advanced COVID-19 disease.

1. Pathophysiology of COVID-19

The clinical signs of COVID-19 are consistent with those observed in viral pneumonia. Abnormal chest CT scans showing bilateral multiple peripheral ground-glass opacities has been observed in 98 % of hospital cases [2]. These pulmonary changes are likely responsible for both systemic and localized immune response leading to a hyperinflammatory state. The mortality rate in patients is suspected to be related to virally driven cytokine storm similar to that seen in SARS-CoV-2 infections. The cytokine storm is a result of a severe immune reaction in the lungs as measured by high levels of inflammatory markers (c-reactive protein, serum ferritin) and cytokine levels (IL-6, IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A, and TNFα) in the plasma [1]. ICU patients had higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A, and TNFα as compared to non-ICU patients, suggesting that the presence of high circulating cytokine levels is associated with the severity of the disease [2,5]. The current urgent need for effective treatment of this life-threatening disease by targeting hyper-inflammation in the lungs, is of high, compelling interest. Although use of corticosteroids might be beneficial in reducing inflammation-induced lung injury, evidence from SARS and MERS patients shows no or little improvement in mortality rates, with a delay in viral load clearance [6,7]. An anti-inflammatory therapy using a monoclonal antibody against IL-6 is currently undergoing phase III trial in COVID-19 patients [8] and could lead to promising results. The effectiveness of this therapy depends on the control of a single factor (IL-6) in this multidimensional process. It is possible that the approach will be limited in efficacy and it might be necessary to interfere with the inflammatory cascade at a higher level (i.e., eliminating the pro-inflammatory efferent pathway) to appropriately control the multimodal aspect of this inflammatory process.

2. Afferent innervation, TRPV1 channel and inflammation in the lungs

The respiratory tract (higher and lower) is densely populated by sensory afferents originating from neurons in the nodose (vagal) ganglia (VG) and dorsal root ganglia (DRG). Many of the neurons in these ganglia express high levels of the transient receptor potential vanilloid 1 (TRPV1) ion channel. The crosstalk between TRPV1 positive nerve fibers and immune cells is critical in mediating inflammation of the airway following exposure to either inhaled allergens or viral infection [13,14]. TRPV1 is strongly implicated in the regulation of irritant-induced airway responses and cough [15]. The expression of TRPV1 positive cells is increased in the airways of patients with chronic cough [18]. Activation of TRPV1 enhances the release of several pro-inflammatory molecules, including substance P (sP), and cytokines such as IL-6. Pro-inflammatory substances have reported to be upregulated in COVID-19 cases and reflect the severity of the disease [16].

A recent study has demonstrated that respiratory viral infections (by rhinovirus, respiratory syncytial virus or measles virus) can upregulate TRPV1 receptors by channel specific mechanisms [17]. This upregulation can drive an inflammatory cascade in the lungs leading to airway hyperactivity and is dependent on the viral load and duration of infection. Interestingly, treatment with TRPV1 antagonist in this study significantly inhibited TRPV1 upregulation post viral infection. The interaction of SARS-CoV-2 viruses with TRPV1 receptors has not yet been investigated but given the respiratory pathophysiology in COVID-19 cases, may exhibit similar mechanisms that can result in sensitizing TRPV1 receptors resulting in hyper-inflamed lungs and associated complications. In accord with the aforementioned studies, Baral P. and colleagues demonstrated the role of sensory neurons expressing TRPV1 receptors in the lungs in a model of lethal MRSA pneumonia. These experiments showed that downregulation of the TRPV1 positive channels provided a protective pulmonary environment associated with neutrophil infiltration, enhanced T cell responses and bacterial clearance. Further, calcitonin gene related peptide (CGRP) released from the TRPV1 nerve endings during infection downregulates innate immune responses. Therefore, ablating TRPV1 positive cells and inhibiting CGRP release resulted in improved survival outcomes in MRSA infected mice, thus exposing TRPV1 as a potential therapeutic target in combating pneumonia and associated mortality [9].

Secondary factors playing an aggravating role in the poor outcome of ventilated patients due to COVID-19 must also be considered. Critically ill patients suffering from ARDS are by-definition hypoxemic, which makes mechanical ventilation and oxygen-therapy a life-saving strategy. Unfortunately, both therapies represent a great challenge due to intrinsic complications. Ventilator-Induced Lung Injury (VILI) has been identified as a potential adverse effect of mechanical ventilation. Repetitive cyclic stretch and the resulting regional overdistention is associated with complications leading to pulmonary edema, barotrauma and further hypoxemia. These complications accelerate the deterioration of the pulmonary function. Michalick et. al. [10] demonstrated the role of TRPV1 as an amplifier of TRPV4-mediated response and lung epithelial barrier failure for VILI. Using a knockout mice model, the authors observed that mechanical stress activating TRPV4 and TRPV1 positive cells amplified $Ca+$ signaling, and that inhibition of these receptors reduced the endothelial $[Ca+]$ response to high pressure inflation. This finding suggests that ablation of TRPV1 positive pathways could be beneficial for the prevention or treatment of ventilator-associated lung injury. Hyperoxic acute lung injury (HALI) as a result of high inspired fraction of oxygen (FiO2) during prolong times, in addition to mechanical ventilation, will increase lung inflammation, microvascular permeability and lung epithelial apoptotic cell death [11]. An initiating factor involved in this type of lung injury is the generation of reactive oxygen species (ROS) that will activate a signaling cascade leading to overproduction of substance P (by TRPV1 activation) with subsequent increase in cytokines levels [12]. The role of TRPV1 positive cells in HALI has been described [12]. Data showing the significantly prolonged survival of TRPV1 knockout mice after prolonged exposure to high FiO2 suggests the crucial role of substance P in the amplification of the inflammatory cascade leading to the deterioration of pulmonary microenvironment and function.

TRPV1 expression in the lungs is upregulated in the face of pathology [24] and can change as individuals age, switching from a mostly anti-inflammatory function in the young to a pro-inflammatory function in the elderly as shown in a systemic inflammatory animal model looking at that precise phenomena [20]. The incidence of fatality associated with older patients, especially people over 65-years-old, might be exacerbated by the pro-inflammatory function of TRPV1 afferent nerves in the lungs. This in addition to a declining immune function in people as they get older making them more susceptive to infections, and the impact of higher comorbidities in the elderly population could be contributing factors and help explain the progression and disproportionate impact of COVID-19 in the elderly.

3. Rationale for blocking afferent signaling (TRPV1) in COVID-19 patients

We suggest that the association of TRPV1 expressing innervation combined with the virally driven hyperinflammation in COVID-19 cases might be the root cause of the lethal aspect of the disease particularly for the elderly. We propose that interrupting TRPV1 signaling might decrease the severity of the acute respiratory distress syndrome present in COVID-19 patients.

Studies in mice, with LPS-induced lung injury have shown that capsazepine (a TRPV1 antagonist) pre-treatment prevented the increase

in respiratory system resistance, tissue damping, and decreased the area of collapsed lung parenchyma [19]. Blocking lung sensory neurons signaling could result in inactivation of efferent system decreasing associated inflammation and cytokine storm adverse effects. Baral P and colleagues have further demonstrated that chemical ablation of pulmonary sensory neurons expressing TRPV1 receptors (by use of a highly potent TRPV1 agonist) dramatically improved survival against MRSA cytokine storm lethal pneumonia in a mouse model of the disease. In the experiments, they had no fatalities in the chemically ablated neuron groups versus an 80% fatality in the untreated control group. Ablation of TRPV1 expressing neurons and the inhibition of associated CGRP release resulted in control of neurogenic inflammation and improved the overall survival outcome in the infected mice [9].

Silencing TRPV1 afferent pathways as part of the treatment for COVID-19-related pneumonia/ARDS will not only address the intrinsic pathophysiology of the viral infection and systemic inflammation, but should also help reduce the complications associated with the current gold-standard therapy (mechanical ventilation/oxygen therapy) in severely compromised patients.

Silencing TRPV1 positive nerve fibers could eventually be also of interest to limit the progression of the disease from mild stages to acute respiratory distress.

4. Resiniferatoxin (RTX) potential to lessen impact of COVID-19 progression

The afferent innervation of the pulmonary system is mainly conducted by the vagal nerve and its branches. TRPV1 expressing C-fibers are small diameter unmyelinated fibers in the vagal nerve and responsible for several processes in the airways and lungs. It is important to note that afferent fibers innervating pulmonary structure are also carried by sympathetic fibers with cell bodies located in the dorsal root ganglia of the thoracic segment between T1 and T6. The activation of this thoracic segment has been related to severe pneumonitis [21].

In consideration of pulmonary neuroanatomy, potential routes of administration for an ablative agent would include thoracic epidural injections, peri-ganglionic nerve block or intra-ganglionic injections for "chemical" targeted lung denervation. For instance, a practical approach for the ablation of vagal afferent/efferent TRPV1 positive fibers is performing an ultrasound guided nerve block targeting the cervical section (pre-thoracic) of the Vagus nerve.

Resiniferatoxin (RTX), a diterpene ester, is an active pharmaceutical ingredient (API) purified from the latex of several cactus-like Euphorbia species. An ultra-potent agonist of the TRPV1 receptor, it works by inducing neurolysis of TRPV1-expressing neurons in dorsal root ganglia (DRG), dorsal horns (DH) of the spinal cord, or peripheral nerve ending when applied locally as a nerve block. The strong binding of resiniferatoxin to TRPV1 receptors forces the opening of the channel gates leading to a slow and sustained increase in intracellular $Ca2+$, which in turn disrupts the intracellular mitochondrial metabolism and results in neural cell or nerve fiber deletion within minutes [25].

Pharmacology safety studies indicate that the resiniferatoxin mechanism of action is limited to those nerves involved with neurogenic inflammation [26]. Central administration of RTX causes permanent neurolysis via apoptosis in contrast to peripheral application which leads to a more transient effect because the peripheral nerve can regenerate. Ablation of the TRPV1 expressing complement of afferent nerves is not associated with effects on motor function or other adverse effects [27] and resiniferatoxin has shown positive impact on functional outcomes in multiple animal pain models.

Multiple toxicology studies performed as IND enabling studies (to allow for human trials) confirm the applicability of resiniferatoxin as a central agent (intrathecal and epidural) or peripheral signal modulation agent (routes validated include intrathecal, epidural, intra-articular, peri-ganglionic, intra-vesicular and as a peripheral nerve block).

Clinical trials currently being pursued for companion animal health have demonstrated the potential for effective application of RTX as an ablative agent utilized peripherally. RTX has been successfully administered to over 200 dogs in multiple studies looking at developing control of arthritis pain by ablating afferent nerve endings to the joints [28]. Also, an exploratory clinical trial in 13 domestic felines demonstrated the drug ability to help control chronic neuropathic limb pain associated with declaw procedures (done years prior), when applied as a perineural nerve block to ablate afferent nerves at the carpal joint level [29].

Multiple human trials for resiniferatoxin indications have recently been completed in the US by Sorrento Therapeutics, and one is still on-going at the National Institute of Health.

A "Phase 1b double-blind study to assess the safety, tolerability and preliminary efficacy of intra-articular administration of resiniferatoxin versus placebo for the treatment of pain due to moderate to severe osteoarthritis of the knee" (clinicaltrials.gov **NCT03542838**) enrolled the last of 94 patients in early January 2020. No dose limiting toxicities were observed at any of the doses administered, which went up to 30 ug per patient administered as a local injection in the knee.

"A Multicenter, Open-Label, Phase 1b Study to Assess the Safety and Define the Maximally Tolerated Dose of Epidural Resiniferatoxin Injection for the Treatment of Intractable Pain Associated with Cancer" (**NCT03226574**) enrolled the last of 17 patients in mid-February 2020, following a dose escalation plan from 0.5ug to 25ug epidural administration. The study completed with no dose limiting toxicities.

We propose to pursue both an epidural / peri-ganglionic application of RTX, and a bilateral vagal nerve block in patients with advanced COVID-19 disease in support of current palliative ventilation therapy, in the hope that ablating afferent nerves at the pre thoracic or DRG level will increase survival of patients progressing towards critical condition. As we have product available, an active IND and have treated about 30 patients through central routes without significant adverse events or dose limiting toxicity, we believe there is enough safety data to justify pursuing an IND for this indication.

Conflicts of interest

The authors are employed (or director) of Sorrento Therapeutics, Inc. which is funding the discovery, development and clinical trials of resiniferatoxin (RTX) described in this review.

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