TRPV4: an exciting new target to promote alveolocapillary barrier function

Rory E. Morty1,2 and Wolfgang M. Kuebler3,4,5

1Department of Lung Development and Remodelling, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; 2Department of Internal Medicine (Pulmonology), University of Giessen and Marburg Lung Center (UGMLC), member of the German Center for Lung Research (DZL), Giessen, Germany; 3Institute of Physiology, Charité Universitätsmedizin Berlin, Germany; 4Departments of Surgery and Physiology, University of Toronto, Toronto, Ontario, Canada; and 5The Keenan Research Center for Biomedical Science of St. Michael’s, Toronto, Ontario, Canada

Submitted 4 September 2014; accepted in final form 17 September 2014

Morty RE, Kuebler WM. TRPV4: an exciting new target to promote alveolocapillary barrier function. Am J Physiol Lung Cell Mol Physiol 307: L817–L821, 2014. First published October 3, 2014; doi:10.1152/ajplung.00254.2014.—Transient receptor potential (TRP) channels comprise a group of nonselective cation channels that currently receive much attention in the cardiopulmonary system, both as pathogenic mediators and as targets for the treatment or prevention of lung and airway disease (17, 25). The TRP channels in mammals are currently organized, on the basis of protein and nucleotide sequence homology, into six families: the canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), mucolipin (TRPML), polycystin (TRPP), and ankyrin (TRPA) families. Of these families, the six-member TRPV family is currently the subject of intense research in respiratory health and disease. Specifically, TRPV1 channels, which are expressed in nonmyelinated afferents innervating the airways and lungs (23), have evolved as novel pathogenic mediators and therapeutic targets in asthma and cough (4, 9, 17, 30, 35, 41). Recently, it has been demonstrated in rats that TRPV1 facilitates the activation of lung vagal C-fiber afferents by cigarette smoke (45), and some demonstrated in rats that TRPV1 facilitates the activation of in asthma and cough (4, 9, 17, 30, 35, 41). Recently, it has been demonstrated that the inhibitors are not applied prophylactically, prior to induction of lung injury, but in a clinically more relevant treatment strategy, either immediately after chlorine exposure, or in case of acid instillation, with a 30-min lag time. Application of TRPV4 inhibitors had potent anti-inflammatory effects in these two injury models, limiting neutrophil and macrophage infiltration and dampening the levels of proinflammatory cytokines and chemokines in the bronchoalveolar lavage fluid and serum. These observations were largely recapitulated in trpv4−/− mice. In the chlorine inhalation model, postinjury application of TRPV4 inhibitors also improved blood oxygenation and reduced airway hyperreactivity. Interestingly, these effects are in part reminiscent of the attenuated inflammatory response and airway hyperresponsiveness in allergen-challenged trpc1−/− mice (49), an idea with increasing relevance in view of the recent identification of TRPV4-TRPC1 heterodimers, which can assemble to form a functional...
store-operated \(Ca^{2+}\) channel (31). The investigators went on to propose that under conditions of lung injury, TRPV4 may be activated by endogenously produced N-acylamine cannabinoids, fatty acid-derived products related to anandamide that can activate various TRP channels, levels of which were found to be elevated in the lungs of chlorine or hydrochloric acid-treated mice. Notably, anandamide has been shown to activate TRPV4 channels in an indirect manner via cytokrome \(P-450\) epoxygenase-dependent formation of epoxyeicosatrienoic acids (EETs) (44). Intriguingly, EETs are also the dominant eicosanoids in human lungs upon microbial challenge and hence are considered to contribute substantially to inflammatory-infectious pulmonary injury. Whether induction of TRPV4-mediated chloroform- and acid-induced lung injury similarly depends on the formation of EETs, or whether lung injury subsequent to microbial infections involves TRPV4, remains, however, to be demonstrated.

This exciting study builds on a solid body of data that supports a role for TRPV4 in a broad spectrum of lung and airway functions and disease processes. TRPV4 has been implicated as a key regulator of lung endothelial barrier integrity, specifically, the integrity of the lung alveolar capillary endothelium, which is most relevant to alveolar flooding associated with acute lung injury (5, 21, 39). TRPV4 activation by the phorbol ester 4α-phorbol 12,13-didecanoate (4αPDD) increases lung capillary permeability and triggers the formation of alveolar edema (1, 46), whereas, conversely, hydrostatic lung edema formation and capillary leakage are reduced in \(trpv4^{-/-}\) mice (24, 50). In lung microvessels, hydrostatic stress activates TRPV4, presumably by circumferential vessel stretch, and the resulting increased endothelial \(Ca^{2+}\) concentration (27) triggers a diverse set of vascular responses. These responses include an increase in endothelial permeability through activation of myosin light chain kinase (50), the stimulation of endothelial NO synthesis (26), and the exocytosis of Weibel-Palade bodies with subsequent expression of P-selectin on the vascular surface (28). Although it should be noted that the latter response is confined to larger lung microvessels but is absent in alveolar capillaries, which are devoid of Weibel-Palade bodies (53) and do not express P-selectin in response to TRPV4 agonists (46), it may nevertheless be of considerable relevance in the context of the present study. Indeed, TRPV4-mediated exocytosis of Weibel-Palade bodies, P-selectin expression, and the concomitant release of chemokines such as interleukin-8 may provide a mechanistic explanation for the strikingly reduced infiltration of inflammatory cells following treatment with TRPV4 inhibitors.

In addition to regulating endothelial permeability and as a result of \(Ca^{2+}\) entry channel function in pulmonary artery smooth muscle cells, TRPV4 also plays an important role in the regulation of lung myogenic tone. Inhibition or deficiency of TRPV4 has been documented to attenuate serotonin-induced \(Ca^{2+}\) elevations in pulmonary artery smooth muscle cells and thus attenuate the contraction of isolated pulmonary arteries (47). A similar role for TRPV4 was recently identified in the lung vascular responses to hypoxia, implicating TRPV4 in the complex signaling cascade that mediates hypoxic pulmonary vasoconstriction. Although this finding is essentially in line with the documented role of TRPV4 in lung vascular remodeling associated with exposure to chronic hypoxia (6, 48, 52), it poses a potential threat to the clinical use of TRPV4 inhibitors for the treatment of acute lung injury. Lung injury is characterized in distribution of a heterogeneous fashion throughout the lungs, and redistribution of blood flow from poorly ventilated to better aerated lung areas by the von Euler-Liljestrand mechanism provides an intrinsic rescue mechanism by which an organism can maintain adequate arterial blood oxygenation even when significant parts of the lung have ceased to participate in gas exchange. Pharmacological inhibition of such a key physiological response would potentially be detrimental to any patient with acute lung disease. The fact that TRPV4 inhibition improved arterial oxygenation in the chloride injury model seems to argue against such an effect, but the situation may differ in scenarios of more heterogeneous injury.

In addition to effects in the pulmonary vasculature, TRPV4 has also been implicated in the maintenance of epithelial barrier function in the lung (38), and TRPV4 mediates the constriction of airway smooth muscle cells in a cysteinyl leukotriene-dependent manner (32). TRPV4 is also expressed in alveolar macrophages (18), implying a potential role in the regulation of inflammation. This has proven relevant in the context of lung injury, since adoptive transfer of macrophages from \(trpv4^{-/-}\) mice to \(trpv4^{+/+}\) mice restored susceptibility of \(trpv4^{-/-}\) lungs to mechanical injury induced by high peak inflation pressure ventilation (19). Given the broad spectrum of roles in lung and cellular function, TRPV4 has been conclusively identified as a mediator of hydrostatic lung edema (24, 50), as well as edema associated with experimental ventilator-induced lung injury (20). These observations have led to studies that have documented the usefulness of TRPV4 inhibition in models of pulmonary edema formation secondary to heart failure, in which application of an earlier-generation TRPV4 inhibitor GSK2193874 decreased extravascular lung water and increased arterial oxygenation (42).

In the background of the emerging role of TRPV4 in cardiopulmonary physiology, the study by Balakrishna and colleagues (2) provides a foundation for further exciting work. Several important questions immediately emerge. A very striking observation made in the report is that TRPV4 inhibitors exhibit potent anti-inflammatory activity, which was comparable to that of glucocorticoids. Both TRPV4 inhibitors employed blocked inflammatory cell influx, dampened myeloperoxidase activity in the bronchoalveolar lavage as a measure of neutrophil infiltration and activation, and blunted proinflammatory cytokine and chemokine production. These effects were recapitulated in \(trpv4^{-/-}\) mice. However, the mechanisms underlying all of these effects are not understood. One possibility discussed above is that TRPV4 inhibitors act primarily on endothelial and epithelial cells, not only preventing changes in barrier function, but also blocking other \(Ca^{2+}\)-dependent processes such as the release of cytokines and adhesion molecules or the facilitation of neutrophil transits (40). Alternatively, do these inhibitors (and thus, TRPV4) directly impact macrophage and neutrophil function and mobility? Functional expression of TRPV4 in macrophages and its central role in the elicitation of ventilation-induced lung injury has been previously documented in isolated lung studies (19). In the present study, Balakrishna and colleagues furthermore analyzed RNAseq neutrophil transcriptome datasets in silico.
and concluded that the frequency of TRPV4 transcripts in either noninduced or induced neutrophils was very low. This notion, however, is in contrast to quantitative real-time PCR analyses from murine neutrophils demonstrating an abundance of TRPV4 mRNA, compared with other TRP channels including TRPC3, TRPC6, TRPC7, and TRPM4 (7). The actual cell type (or types), through which TRPV4 inhibitors exert their anti-inflammatory effects remains, hence, so far unclear and may require extensive work using adoptive transfer models and chimeric mice. The general notion, however, of a key role for TRPV4 in inflammatory disease processes is substantiated by recent reports that TRPV4 blockade can effectively protect against experimental colitis in mice (12), whereas, conversely, TRPV4 agonists trigger joint inflammation in rats (10). However, this anti-inflammatory potential also bears a serious risk for the therapeutic use of TRPV4 inhibitors in lung injury: the majority of patients with acute respiratory distress syndrome suffer from concomitant pulmonary (pneumonia) or systemic

Fig. 1. Role of TRPV4 in acute lung injury. Inhalation or intratracheal instillation of injurious chemicals such as chlorine gas or acid causes acute lung injury that is characterized by influx of inflammatory cells and failure of the alveolocapillary barrier, resulting in the formation of a cell- and protein-rich alveolar edema. Pharmacological inhibition or genetic deficiency of TRPV4 alleviates these effects, demonstrating that TRPV4, which is expressed on various lung parenchymatous and inflammatory cells, contributes critically to this scenario: In lung capillary endothelial cells (EC), TRPV4 activation increases vascular permeability, thus promoting protein and fluid leak. In extra-alveolar vessels, TRPV4 activation may stimulate endothelial P-selectin expression and nitric oxide (NO) synthesis. In pulmonary artery smooth muscle cells (PASMC), TRPV4 mediates vasoconstriction and may thus modulate ventilation/perfusion matching in injured lungs. In alveolar macrophages, TRPV4 activation results in the formation of reactive oxygen species (ROS) and NO, which in combination give rise to peroxynitrite, causing tyrosine nitrosylation of proteins. TRPV4 has also been reported to be expressed on alveolar epithelial cells and, according to some authors, neutrophils, yet their contribution to TRPV4-mediated acute lung injury remains to be resolved. The wide expression and multifold functions of TRPV4 in the lung render this channel a promising yet at the same time complex target for the treatment of acute lung injury. PMN, polymorphonuclear cell, neutrophil; ATII/ATIII cell, alveolar type 1/type 2 cell.
TRPV4 blockade may represent a double-edged sword. The including a role in lung vasomotor control, the inflammatory TRPV4, and the multitude of functions ascribed to TRPV4, clinical conditions. Because of the ubiquitous expression of as promising therapeutic strategy for the treatment of related lung injury (outlined in Fig. 1) and identifies TRPV4 inhibitors to be assessed the responses to (and protection against) chlorine exposure in other mouse strains, which are notorious for strain-dependent variability in response to chlorine inhalation (14, 33).

The combination of vascular protective effects and anti-inflammatory properties also make TRPV4 inhibitors interesting candidates for investigation in animal models of pulmonary vascular disease with an inflammatory component, such as pulmonary arterial hypertension. TRPV4 has been directly implicated in pulmonary vascular remodeling, in response to chronic exposure of mice to hypoxia (47), a well-characterized animal model of pulmonary arterial hypertension (15, 43); however, perivascular inflammation is not appreciable in this model. In contrast, inflammation is a key player in the related rat monocrotaline model of pulmonary arterial hypertension (16). It would be interesting to explore the effect of TRPV4 inhibitors in that and other animal models of pulmonary disease with vascular and inflammatory components (37), particularly considering that pulmonary hypertension is a frequent complication of acute lung injury (36).

The present work by Balakrishna and colleagues (2) identifies a novel pathogenic role for TRPV4 that extends its previously reported relevance in cardiogenic lung edema and ventilator-induced lung injury to scenarios of chemically induced lung injury (outlined in Fig. 1) and identifies TRPV4 inhibitors as promising therapeutic strategy for the treatment of related clinical conditions. Because of the ubiquitous expression of TRPV4, and the multitude of functions ascribed to TRPV4, including a role in lung vasomotor control, the inflammatory response, and the regulation of systemic blood pressure, TRPV4 blockade may represent a double-edged sword. The therapeutic benefits of TRPV4 inhibition will have to be carefully weighed against potential adverse effects on a case-by-case basis.

GRANTS

This work was supported by the Max Planck Society (R. E. Morty); by the German Centre for Lung Research (to R. E. Morty); by research grant 62589035 from the University Medical Center Giessen and Marburg (to R. E. Morty); by the Federal Ministry of Higher Education, Research, and the Arts of the State of Hessen LOEWE-Programm (to R. E. Morty); and by the German Research Foundation through Individual Grant Mo1789/1 (to R. E. Morty) and Excellence Cluster 147 Cardio-Pulmonary System (to R. E. Morty). W. M. Kuebler received support from the Canadian Institutes of Health Research, the German Research Foundation, and the Heart & Stroke Foundation of Canada.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.E.M. and W.M.K. analyzed data; R.E.M. and W.M.K. interpreted results of experiments; R.E.M. and W.M.K. drafted manuscript; R.E.M. and W.M.K. edited and revised manuscript; R.E.M. and W.M.K. approved final version of manuscript.

REFERENCES

Depletion of intracellular Ca\textsuperscript{2+} via the TRPV6 receptor and the calpain pathway. Dasgupta P.

Am J Physiol Lung Cell Mol Physiol


