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Most recently, in a scientific collaboration effort between APEPTICO, the Vascular Biology Center of the Georgia Regents University (Prof. Dr. Rudolf Lucas, USA), and the Institute of Pharmacology and Toxicology of the University of Vienna (Prof. Dr. Rosa Lemmens-Gruber, Austria), the detailed mechanism has been discovered how APEPTICO’s clinically stage “AP301-peptide” (identical to the lectin-like domain of native TNF-alpha) activates the specific pulmonary ion channel “ENaC” to result in “Activation of Lung Oedema Clearance”.

In the most recent EDITORIAL in “American Journal of Respiratory Critical Care” (Am J Respir Crit Care Med Vol 190(6), pp 595–605, Sep 15, 2014; http://www.atsjournals.org/doi/abs/10.1164/rccm.201407-1364ED#.VBfyMha7ZbI) by Gary C. Sieck and Mark E. Wylam from the Mayo Clinic, Rochester, both the ground-breaking scientific discoveries and the clinical-therapeutically potential of AP301 peptide have been highlighted.

In the EDITORIAL the authors summarise as follow “Previously, the lectin-like domain of TNF-alpha was shown to activate ENaC in type 2 alveolar epithelial cells (Ref. 1). Importantly, this effect has been mimicked by a small 17-amino acid circular peptide known as TNF inhibitory peptide (TIP; APEPTICO code “AP301”). A recent European study compared placebo treatment with TIP inhalation in patients with ALI/ARDS and found that TIP elicited earlier and more pronounced clearance of pulmonary edema (Ref. 2, Ref. 3). The molecular mechanism underlying TIP-induced ENaC activation remained uncertain until the study by Czikora and colleagues, published in the September 1, 2014, issue of the Journal (Ref. 4).”

The EDITORIAL makes explicate reference to APEPTICO’s most recently completed phase IIa clinical study in patients with ARDS and pulmonary oedema. During the European Respiratory Society International Conference (Munich, 6-9 September 2014), Dr. Krenn from the Department of Intensive Care Medicine, Medical University of Vienna, reported key findings of the AP301-trial in mechanically ventilated patients with pulmonary permeability oedema.

The EDITORIAL concludes, “Taken together, these basic science results provide new physiological insight into the potential role of the lectin-like domain of TNF-a (AP301 peptide) and support the novel therapeutic use of AP301 aerosols in patients with ALI/ARDS and ischemia reperfusion lung injury.”

In addition, Dr. Krenn’s presentation of the AP301 clinical trial at the ERS International Conference was immediately reflected as “Top story of the week” in Pulmonary/Respiratory Medicine (http://dgnews.docguide.com/novel-peptide-activates-pulmonary-oedema-clearance-mechanically-ventilated-patients). MUNICH, Germany -- September 10, 2014: Jenny Power reported “Novel Peptide Activates Pulmonary Oedema Clearance in Mechanically Ventilated Patients. -- Acute lung injury (ALI) mediated by acute pulmonary permeability oedema can be reduced by a novel synthetic peptide that promotes pulmonary oedema clearance by decreasing extra-vascular lung water (EVLW) among mechanically ventilated patients in the intensive care unit (ICU).” (PDF copy attached).

Dr. Bernhard Fischer, CEO of APEPTICO added: ”We are very proud that Prof. Rudolf Lucas’ scientific discoveries and APEPTICO’s therapeutic approach have been rewarded an Editorial in the American Journal of Respiratory Critical Care”. “APEPTICO develops the “AP301-peptide”, a synthetic version of the lectin-like domain of TNF, as a new life-saving medicine for patients with pulmonary permeability
oedema. Most recently we could demonstrate in a clinical phase II proof-of-concept study (ClinicalTrials.gov ID NCT01627613) that orally inhaled “AP301-peptide” activates alveolar liquid clearance in mechanically ventilated patients having lung oedema and ARDS,” he added.

Reference


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About APEPTICO GmbH (www.apeptico.com)
APEPTICO is a privately-held biotechnology company based in Austria, developing peptide-based products targeting chronic and life-threatening diseases. The peptide molecules correspond to validated, pharmacodynamic active structures and domains of well-known proteins and biopharmaceuticals. By concentrating on synthetically produced protein structures APEPTICO avoids general risks associated with gene- and cell-technologies. APEPTICO makes use of its technology platforms PEPBASE(TM) and PEPSCREEN(TM) to significantly reduce cost and to shorten time to market.

About the AP301-peptide / TIP-peptide
The AP301-peptide (synonym to TNF-derived TIP-peptide) is a synthetic molecule whose structure is based on the lectine-like domain of the human Tumour Necrosis Factor α. The AP301 peptide is water soluble and can be administered into the lung by oral inhalation. Formulated AP301 is easily nebulised and the resulting aerosol is composed of peptide/water droplets of diameter 4 μm or less. APEPTICO’s most recent clinical phase II proof-of-concept study (ClinicalTrials.gov ID NCT01627613) demonstrated that orally inhaled AP301-peptide activates alveolar liquid clearance in mechanically ventilated patients with pulmonary permeability oedema and ARDS.

Comprehensive research and development studies conducted by Dr. Rudolf Lucas and the APEPTICO research consortium demonstrated, that AP301-peptides are effective therapeutic molecules in various forms of pulmonary oedema, such as pulmonary permeability and hydrostatic oedema, high altitude pulmonary oedema (HAPE), pulmonary oedema associated with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), pulmonary oedema resulting from pneumonia, sepsis and lung transplantation (primary graft dysfunction).

APEPTICO’s AP301 has been granted orphan drug status (i) for treatment of pulmonary permeability oedema in ALI/ARDS, (ii) for treatment of primary graft dysfunction following lung, and (iii) for treatment of high altitude pulmonary oedema by the European Commission and European Medicines Agency (EMA) and by the Food and Drug Agency (FDA).

About pulmonary oedema
Pulmonary oedema occurs when fluid leaks from the pulmonary capillary network into the lung.
interstitium and alveoli. There are many possible causes of lung oedema, such as heart failure (cardiac/hydrostatic lung oedema); inhaling high concentrations of smoke, toxins, or oxygen; severe burns; blood infections / sepsis; infection of the lung / pneumonia; aspirations, cerebral damage or trauma to other parts of the body and lung transplantation. Lungs contain alveoli, which are tiny air sacs where the oxygen is passed into the blood. During lung oedema, blood and fluid begin to leak into the alveoli. When this happens, oxygen cannot enter the alveoli, which means oxygen no longer passes into the blood. Because the lungs are inflamed and filled with fluid, the patient finds it increasingly difficult to breathe. The mortality rate of patients with pulmonary oedema in ALI/ARDS is 35% to 45% within two to four weeks. Currently, no specific drug treatment exists for patients suffering from pulmonary permeability oedema and patients having ARDS. ARDS is also a major economic burden to hospitals and health care budgets. It is estimated that due to a long ICU and hospital stay the cost of every saved live from ARDS is approximately $70,000 USD.

Contact
Univ.-Doz. Dr. Bernhard Fischer, Chief Executive Officer
APEPTICO Forschung und Entwicklung GmbH
Mariahilferstraße 136, Top 1.1.5
1150 Vienna, Austria
T: +43-664-1432919
F: +43-1-2533037795
E-mail: b.fischer@apeptico.com
URL: www.apeptico.com
Paradoxical Use of Tumor Necrosis Factor in Treating Pulmonary Edema

In the normal lung, alveolar fluid clearance is tightly regulated, but in disease states, increased alveolar fluid can arise through alterations in Starling forces, including hydrostatic fluid forces (high-pressure edema), increased filtration coefficient, or reduced reflection coefficient, leading to epithelial permeability (low-pressure) edema. Regardless of the cause, alveolar edema elicits mechanical (decreased lung compliance) and physiologic (hypoxemia) perturbations that can be disastrous for a patient, leading to respiratory failure.

Most research in this important area has focused on the etiology and pathophysiologic of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), which occurs when an infectious or traumatic event (pulmonary or extrapulmonary) causes release of inflammatory mediators and neutrophil accumulation that affects the microcirculation and alveolar epithelial layer of the lung, leading to pulmonary edema. ALI may also occur as a result of ischemia-reperfusion injury after lung transplantation and, occasionally, cardiopulmonary bypass.

The cornerstone of therapies addressing low-pressure pulmonary edema focuses on altering hydrostatic forces, using diuretics and positive end-expiratory pressure. In the National Institutes of Health/ARDS Network-funded multicenter Fluids and Catheters Treatment Trial (FACTT), conservative compared with liberal fluid administration in patients with ALI/ARDS with the intent to limit pulmonary edemagenesis increased the number of days free from mechanical ventilation. Survival was similar with both approaches, but survivors managed using the conservative approach were liberated from the mechanical ventilator 3.2 days sooner. Matthay and others reported that in patients with ALI/ARDS, mean alveolar fluid clearance is only 6% per hour compared with 13% per hour in patients with hydrostatic edema (1). A comparison of clinical management strategies showed that reabsorption of pulmonary edema fluid from the alveolar space is necessary for resolution of ALI/ARDS, and that those patients with better maximal fluid clearance have lower mortality and spend less time on mechanical ventilation (2).

Absorption of Na⁺ ions via apical epithelial sodium channels (ENaC) is essential for water homeostasis in the lung. Transepithelial Na⁺ transport initiated at the apical surface by ENaC and accomplished by Na⁺/K⁺-ATPase drives water absorption from the alveolar surface. Thus, activation of ENaC provides an important therapeutic strategy for restoring lung water homeostasis. Toward this end, it is known that activation of ENaC is regulated by cyclic AMP. Initial findings from the Beta-Agonist Lung Injury Trial (BALTII) (3) suggested that enhanced intracellular cyclic AMP, stimulated by intravenous salbutamol (albuterol), accelerates the resolution of alveolar edema in adult patients with ALI/ARDS. However, a follow-up trial (BALTII2) (4) was stopped early because of safety concerns, noting increased mortality in the treatment group. In a more recent randomized, placebo-controlled clinical trial on more than 280 patients with ALI/ARDS, aerosolized albuterol was found to be ineffective in improving clinical outcomes (5). In addition, because of its off-target effects, albuterol enhancement of intracellular cyclic AMP may not be the best therapeutic approach to promote ENaC activation in patients with ALI/ARDS.

Might there be another way to increase ENaC activity? With respect to the pathophysiology of ALI/ARDS, we often think of inflammatory cytokines such as tumor necrosis factor α (TNF-α) as central culprits in mediating the negative effects of inflammation, and thus, as part of the pathophysiological sequelae serving to worsen the symptoms of ARDS. Levels of TNF-α are elevated in patients with ARDS, and both activation of TNF-α receptors in the pulmonary microvasculature (endothelial and smooth muscle cells) and alveolar epithelial cells appear to mediate, at least in part, the dysregulation of alveolar fluid clearance and increased microvascular permeability underlying pulmonary edema in ARDS. Previously, the lectin-like domain of TNF-α was shown to activate ENaC in type 2 alveolar epithelial cells (6). Importantly, this effect has been mimicked by a small 17-amino acid circular peptide known as TNF inhibitory peptide (TIP; also named AP301). A recent European study compared placebo treatment with TIP inhalation in patients with ALI/ARDS and found that TIP elicited earlier and more pronounced clearance of pulmonary edema (7, 8). The molecular mechanism underlying TIP-induced ENaC activation remained uncertain until the study by Czikora and colleagues, published in the September 1, 2014, issue of the Journal (9).

Their study provides evidence for a novel, nonreceptor-mediated mechanism by which the TIP activates ENaC, and thereby promotes lung alveolar fluid clearance. Specifically, these investigators systematically explored the effects of a synthetic TIP peptide, which mimics the lectin-like (non-TNF-α receptor) domain of TNF-α. In a series of experiments employing a variety of techniques (transgenic triple mTNF knock-in mice expressing mutant TNF-α, molecular biology, biochemistry, and electrophysiology), the investigators provide convincing evidence for a TNF-α (TIP)-mediated mechanism for direct ENaC activation and rectification of both hydrostatic and permeability-based pulmonary edema. They show that TIP directly binds to the intracellular carboxy-terminal of the α subunit of ENaC, thereby increasing the channel open probability and enhancing Na⁺ absorption.

They also show that TIP binding maintains expression of the α subunit of ENaC and stabilizes the channel structure through interactions with myristoylated alanine-rich C-kinase substrate and phosphatidylinositol 4,5-bisphosphate that are essential for preserving the open configuration in the presence of pore-forming bacterial toxins.
Finally, they developed a novel triple mTNF knock-in mouse model, in which "triple" refers to the substitution of nonsense nucleotides encoding for three distinct amino acids that are normally required for the Na⁺ uptake stimulatory activity of TNF-α, the so-called functional alveolar liquid clearance-stimulatory domain. The authors show that when these triple mTNF knock-in mice were exposed to pneumococcal cholesterol binding pore-forming toxin, a model of pulmonary edema, there was no change in the quantal generation of TNF-α in the bronchoalveolar lavage fluid, but there was reduced ENaC activity, decreased ENaC-α protein expression, and greater lung edema. This finding suggests a physiological role for the lectin-like domain of native TNF-α in alveolar fluid clearance and the resolution of pulmonary edema. Taken together, these basic science results provide new physiological insight into the potential role of the lectin-like domain of TNF-α and support the novel therapeutic use of TIP aerosols in patients with ALI/ARDS and ischemia reperfusion lung injury.

Author disclosures are available with the text of this article at www.atsjournals.org.

Gary C. Sieck, Ph.D.
Department of Physiology and Biomedical Engineering
Mayo Clinic
Rochester, Minnesota

Mark E. Wylam, M.D.
Division of Pulmonary and Critical Care Medicine
Mayo Clinic
Rochester, Minnesota

References


Search for Distinct Mechanisms in Eosinophilic and Noneosinophilic Airway Inflammation

Chronic rhinosinusitis (CRS) is an inflammatory disease of the upper respiratory tract affecting up to 30 million Americans annually. It is associated with a significant impairment in quality of life and places a large financial burden on the healthcare system, with more than $6 billion spent annually on management (1–4). CRSwNP, a subset of CRS, is characterized by the presence of nasal polyps and chronic inflammation of the sinonasal mucosa. In European and American patients, CRSwNP is characterized by type 2 inflammation and eosinophilia. However, there is accumulating evidence, especially in China, that almost half of patients with CRSwNP in Asian countries have a noneosinophilic pattern of inflammation in their polyp tissue that is characterized by a mixed type 1 and/or type 3 response (5, 6). Although the mechanisms that drive these phenotypes are unclear, it has been suggested that differences in Th cell subsets found in polyps from eosinophilic and noneosinophilic patients may play an important role (7). Dendritic cells (DCs) are known to be important in skewing Th responses in the mucosa (8), and thus may be important for skewing Th cells in polyps. However, there has been a lack of in-depth analyses of Th cell subsets found in polyps from different CRSwNP groups, and few studies have investigated the importance of DCs in CRSwNP pathogenesis (9, 10).

In this issue of the Journal (pp. 628–638), Shi and colleagues evaluated the function and phenotype of Th and DC subsets from polyps of eosinophilic and noneosinophilic patients with CRSwNP in China to assess any differences (11). Interestingly, many of the features examined in the Th and DC subsets isolated from polyps did not differ between the two groups of patients with CRSwNP. The researchers found similar elevations of IL-17A⁺ and IFN-γ⁺ CD4⁺ cells in polyps from both groups compared with controls, confirming a recent study from Europe (12). Likewise, they found similar elevations of activated DC subsets (both myeloid DC [mDC] and plasmacytoid DC [pDC]), and these DCs produced equivalent elevated levels of IL-6 and...
Novel Peptide Activates Pulmonary Oedema Clearance in Mechanically Ventilated Patients

By Jenny Powers

MUNICH, Germany -- September 10, 2014 -- Acute lung injury (ALI) mediated by acute pulmonary permeability oedema can be reduced by a novel synthetic peptide that promotes pulmonary oedema clearance by decreasing extravascular lung water (EVLW) among mechanically ventilated patients in the intensive care unit (ICU).

The findings were presented here at the European Respiratory Society (ERS) 2014 Annual Congress by Katherina Krenn, MD, Medical University of Vienna, Vienna, Austria.

For the study, 20 patients on mechanical ventilation received inhaled aerosolised AP301 every 12 hours for 7 days and 20 patients received inhaled saline. Reduction of pulmonary oedema -- the primary endpoint -- was determined by measurement of EVLW content twice daily.

Pulmonary oedema clearance was more pronounced in patients with higher sequential organ failure assessment (SOFA) scores receiving AP301. No statistically significant change was observed in patients with SOFA scores <11; however the small sample size may have contributed to this result.

By day 7, patients with SOFA scores ≥ 11 receiving AP301 showed a reduction from baseline of -37.5 in EVLW versus -7.9 among patients receiving placebo (P< .05).

The researchers also assessed the partial pressure of arterial oxygen and fraction of inspired oxygen (PaO2/FiO2 100/200) ratio daily. The PaO2/FiO2 100/200 change from baseline among patients with SOFA scored receiving AP301 was -35.0 versus -1.2 among patients receiving placebo.

Measurements of the oxygenation Index, Murray Lung Injury scores, and Peak Airway Pressure all favoured patients treated with AP301 over placebo.
Patients in the higher SOFA stratum receiving AP301 achieved 20 ventilator-free days compared with 13 ventilator-free days for the placebo group within the 28-day trial period.

A total of 30 adverse events (AEs) were reported by more than 10% of patients in the AP301 group compared with 31 AES in the placebo group. The most common AEs with AP301 were anaemia, thrombocytopenia, and tracheostomy. The most common reported AEs with placebo were tracheostomy, atrial fibrillation, and fever. By day 28, 25% of the patients had died (6 receiving AP301 and 4 on placebo).

Funding for this study was provided by Apeptico Research and Development.

[Presentation title: Oral Inhalation of AP301 Peptide Activates Pulmonary Oedema Clearance: Initial Results From a Phase 2a Clinical Trial in Mechanically Ventilated ICU Patients. Abstract 1386]