Lungs Response To Ventilators Like An Infection

When hospital patients need assistance breathing and are placed on a mechanical ventilator for days at a time, their lungs react to the pressure generated by the ventilator with an out-of-control immune response that can lead to excessive inflammation, new research suggests. While learning that lungs perceive the ventilation as an infection, researchers also discovered potential drug targets that might reduce the resulting inflammation - a tiny piece of RNA and two proteins that have roles in the immune response.

Using human cell cultures, Ohio State University researchers determined that mechanical pressures trigger an innate immune response - the same immune response that the body launches to begin its fight against any kind of infection. The rhythmic pressure of ventilation stimulated the production of pro-inflammatory chemicals by activating proteins called toll-like receptors (TLRs) in lung cells, the research showed.

"We showed that these cells respond to a mechanical force, pressure, as if it were an inflammatory stimulus. They almost perceive it as a bacterial toxin - and they don't like it," said Samir Ghadiali, associate professor of biomedical engineering at Ohio State and co-lead author of the study. "We didn't know until this study how we could possibly turn off that type of mechanically induced inflammation. Essentially what we found are two things that may help: microRNAs and two TLRs in the innate immunity pathway."

The job of TLRs is to recognize distinctive characteristics of pathogens and send out signals to activate other players in the immune system. This response persists during ventilation even with no pathogen present, and the prolonged inflammation can lead to organ failure. Acute lung injuries requiring mechanical ventilation lead to more deaths annually than do breast cancer and prostate cancer combined. The study also suggested that at least one tiny piece of RNA - called a microRNA - is influential in this immune response because its behavior regulates the activation of the toll-like receptor proteins. The findings suggest that manipulating levels of either the microRNA or TLRs could be the basis for potential new treatment options for acute lung injury that requires ventilation.

This study appears online and is scheduled for future print publication in the *Journal of the Federation of American Societies for Experimental Biology (FASEB Journal)*. The study has clinical relevance as well as an influence on the big picture of the field of mechanotransduction - the study of how a mechanical force gets changed into a chemical stimulus inside the body. Mechanics play a role in many disease processes, including hardening of the arteries, other cardiac disorders, kidney problems, emphysema and chronic obstructive pulmonary disease, and cancer.

"This work suggests that there may be a ubiquitous inflammatory mechanism that cells have to respond to anything they perceive as a danger," Ghadiali said. "At least in the lung, we now know the innate immunity pathway plays an important role, and this study showed that a mechanical force gets converted into cytokine production through this well-known pathway." Scientists have known for more than a decade that mechanical ventilation causes inflammation in the lungs that lingers after an acute illness has been cleared, but they have had a hard time figuring out why the lungs respond in this way.

Research attempts to reduce lung damage by altering the force of the ventilation pressure, surface tension or breathing rhythm haven't worked out, said Ghadiali, also an investigator in Ohio State's Davis Heart and Lung Research Institute. So his team, co-led by Patrick Nana-Sinkam, associate professor of internal medicine, sought to determine if there might be another way to regulate lung inflammation in ventilated patients.

The researchers first characterized inflammatory effects of mechanical pressure in experiments using human small airway cells from deep inside the lungs, where ventilation pressure tends to do the most damage. These tests showed that as little as four hours of exposure to ventilation pressure and breathing rates that mimic the clinical environment raised levels of three proinflammatory cytokines, which are chemical messengers that promote an inflammatory response. "The cells keep secreting compounds to recruit the immune system to fight an infection, but there is no infection. It's a response we don't want," Ghadiali said.

The scientists then tested these same cells to examine what role microRNAs (miRs) might have in the lung's inflammatory response to ventilation. Using powerful computers to screen the cells for which miRs responded to ventilation pressure and cyclic breathing patterns, they identified a clear target for more study: a microRNA known as miR-146a. This microRNA's activation increased in the cells as experimental levels of cyclic ventilation pressure increased.

MicroRNAs are small pieces of RNA that bind to messenger RNA, which contains the instructions for building proteins in the process of gene expression. When that connection is made, however, the microRNA has an inverse relationship to its target protein, meaning an overexpressed microRNA leads to lower levels of the protein that the microRNA regulates. "We know that these tiny molecules have the ability to regulate fundamental biological processes in disease. However, their role as mediators of mechanically induced inflammation has yet to be explored," Nana-Sinkam said.

The identification of miR-146a led the researchers to explore the role of two toll-like receptor proteins in the inflammatory response to mechanical ventilation. Because miR-146a has been studied for several years, it is known to target two toll-like receptor proteins that in turn are known to regulate production of the proinflammatory cytokines that the lung cells were found to secrete in response to mechanical pressure.

With this relationship established, the researchers tested the effects of

manipulating these various players on the lung cells' inflammatory response. Among their key findings: Dramatically driving up levels of miR-146a in human small airway cells under mechanical ventilation conditions lowered the activity of the two TLRs, which in turn led to very little cytokine secretion - meaning inflammation was held at bay.

"So we have a microRNA that we can overexpress that targets the innate immunity pathway and shuts down mechanically induced inflammation," Ghadiali said. But the miR is not the only potential target, he noted. Therapies could be designed to target the toll-like receptor proteins themselves, as well, to reduce lung inflammation created by both a disease and the ventilation.

The researchers plan to test the effectiveness of experimental drugs that act on these biological targets in upcoming studies on rodents.