Nicotinic receptor could be target for treatment of lung inflammation

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Camila Maximo Prado, scientist from the Health & Society Institute of the Federal University of São Paulo (UNIFESP) and principal investigator of the study. Credit: Agencia FAPESP

It has been amply proven that smoking can cause serious diseases such as emphysema and cancer. A new study, however, shows that pharmacological stimulation of a specific type of nicotinic receptor in cells of the immune system could be a strategy to treat inflammatory

lung disease.

"In tests with animals, specific stimulation of alpha-7 nicotinic receptors with an experimental drug called PNU-282987 reduced inflammation in a chronic allergic condition similar to asthma and in a lung inflammation model similar to acute respiratory distress syndrome (ARDS), a severe form of respiratory failure that occurs when fluid fills the air sacs in the lungs and that is usually associated with an infectious process," said Camila Prado, principal investigator from the Federal University of São Paulo (UNIFESP), in Brazil.

In this case, the therapeutic effect is associated with activation of nicotinic receptors of the alpha-7 subtype in macrophages, first-line immune system cells that are central to the inflammatory response to a potential threat. Tests showed that mice induced with lung inflammation had improved lung function when treated with the drug, which the researchers attribute to a greater number of tissue-repairing macrophages in comparison with macrophages that release pro-inflammatory molecules.

Both nicotinic and muscarinic receptors are part of the cholinergic system, a branch of the nervous system in which acetylcholine is the main neurotransmitter, Prado explained. In the lungs, acetylcholine is known for its role in bronchoconstriction (acute narrowing of the airways). The active ingredients in several medical drugs used to treat asthma and chronic obstructive pulmonary disease (COPD) are substances that prevent acetylcholine from binding to muscarinic receptors.

However, in a previous study done in collaboration with scientists from the University of Western Ontario, Canada, the research group observed that mice that had been genetically modified to suppress vesicular acetylcholine transporter (VAChT), a protein that mediates the release

of acetylcholine at synapses, presented an exacerbated inflammatory response in the lungs, even without any kind of disease or allergy.

According to Prado, studies suggest that acetylcholine has a protective effect on the lungs that is linked to activation of nicotinic receptors.

"The release of acetylcholine fell 75 percent in these mice, and as a result, they experienced inflammation and airway remodeling similar to that seen in people with asthma. In addition, the cellular signaling pathways involved in the pulmonary inflammatory response were altered."

Based on these findings, the group at UNIFESP decided to test the hypothesis that stimulating the cholinergic system with a drug that binds to nicotinic receptors might attenuate inflammation in the lungs of mice that had not been genetically modified to suppress VAChT.

The first tests were performed using a classic mouse model of acute lung injury. The researchers injected bacterial lipopolysaccharide (LPS), a toxin extracted from the outer membrane of Gram-negative bacteria, into each mouse's trachea.

"About 30 minutes before LPS injection, some of the mice were treated with PNU-282987, a compound that stimulates the alpha-7 nicotinic receptors. Another group was treated with the same compound six hours after LPS injection, when the inflammation reached its peak. In both cases, we observed a significant reduction in the inflammation compared with untreated mice," Prado said.

Besides reducing pulmonary edema (lung swelling), the therapy decreased immune cell release of pro-inflammatory molecules such as interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Analysis of bronchoalveolar lavage fluid (saline

instilled into the lung and then aspirated) showed a reduced presence of immune cells, especially neutrophils and macrophages.

"We also evaluated the effect of this drug on isolated lung macrophages and found a reduced percentage of M1 macrophages, which have a pro-inflammatory profile. At the same time, there was a higher proportion of M2 macrophages, which are associated with the repair of damaged tissue. This may explain the observed improvement in the lung function of the treated mice," Prado said.

Finally, their analysis of lung tissue showed that the treatment reduced activation of the protein NF-kB, a transcription factor that stimulates the production of inflammatory molecules by the cells of the immune system.

According to Prado, the treatment also had positive effects in the chronic inflammation model, which used a classical asthma induction method. The procedure consists of sensitizing the immune system with two injections of ovalbumin, the main protein in egg white, associated with an adjuvant substance that boosts the immune response to this antigen. After 21 days, the animals inhaled the same protein against which their immune system had now developed antibodies at four different times.

"In this model, the animal develops a chronic inflammatory response that eventually leads to a remodeling of the airways," Prado explained. "Collagen is deposited in the airways, and mucus-producing and smooth muscle cells become hypertrophied. All these factors associated with the chronic inflammatory response result in loss of lung function."

Some of the mice were treated with PNU-282987 from the 21st day after the first injection of ovalbumin, at the same time as the inhalatory challenges with the antigen began. The substance was administered by

intraperitoneal injection for seven days.

"We observed a reduction in the lung remodeling process and found that the bronchoalveolar lavage fluid contained a reduced amount of eosinophils, the main type of immune cells associated with asthma," Prado said.

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