Medical Xpress

Study reveals how saline solution can inhibit replication of SARS-CoV-2

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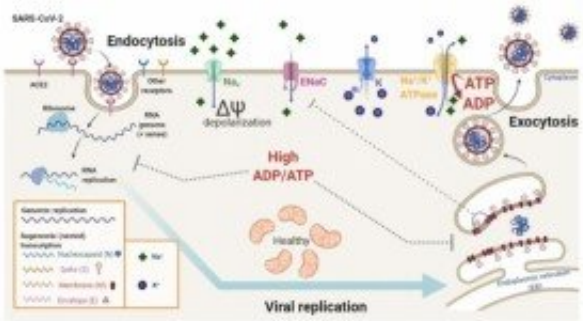

The use of a hypertonic solution of sodium chloride at 1.1% reduced viral replication by 88% in tests involving infected lung cells conducted by researchers at the University of São Paulo. If found efficacious in clinical trials, the strategy could contribute to the development of novel prophylactic interventions and therapies for COVID-19. Credit: Researchers' archive

Researchers at the University of São Paulo (USP) in Brazil have shown that a hypertonic saline solution inhibits replication of SARS-CoV-2, the virus that causes COVID-19, and have elucidated the biochemical mechanism involved. An article reporting the research is published in *ACS Pharmacology & Translational Science*. The study was performed in the laboratory using human epithelial lung cells infected with the virus.

If the strategy proves effective in clinical trials, it could contribute to the development of novel prophylactic interventions to prevent COVID-19 or even treatments for the disease.

"Given the gravity of the pandemic, we believe it would be important to extend this line of research by conducting clinical trials designed to verify the efficacy of using a spray with hypertonic sodium chloride [NaCl] saline as a form of prophylaxis, helping to stop the virus from spreading within the infected organism and reducing the likelihood of more severe inflammation," said Cristiane Guzzo, last author of the article and a researcher at the university's Biomedical Sciences Institute (ICB-USP).

The study was supported by FAPESP. Edison Durigon, Professor of Virology at ICB-USP, and Henning Ulrich, at the Institute of Chemistry (IQ-USP), also participated.

Although the evidence suggests the use of saline inhibits viral replication , it does not afford full protection against infection, let alone a cure. "It's very simple and cheap. It's already used prophylactically against other respiratory diseases, and it could minimize the severity of COVID-19 by reducing viral load. It could be added to safety protocols without replacing the use of face coverings, social distancing and vaccination," Guzzo said.

The right concentration

By comparing different concentrations of the product, the researchers found that a 1.5% NaCl solution completely inhibited viral replication in Vero cells. In human epithelial lung cells, a 1.1% solution was sufficient to achieve 88% inhibition. Vero cells are derived from kidney epithelial cells extracted from an African green monkey, and widely used as a model for studying SARS-CoV-2.

Hypertonic saline is already adopted prophylactically to manage cases of influenza, bronchiolitis, rhinitis, sinusitis, and other respiratory disorders. A spray is sufficient for the upper airways, while a nebulizer

is needed to reach the lungs. These interventions can minimize the symptoms of such diseases, but the mechanisms underlying their effects are poorly understood.

"Our explanation of this intracellular response to the hypertonic solution was basic science but the findings of the study have evident applications in healthcare and clinical approaches to the management of various respiratory diseases," Ulrich said. "What we observed with regard to SARS-CoV-2 is likely to apply to other viruses as well, since the mechanism concerned is part of the host cell's response to infection."

No energy

To grasp the mechanism involved, it is useful to bear in mind that viruses use elements of the host cell such as proteins and energy sources to replicate their genetic material and invade other cells and organs. "We discovered that NaCl doesn't interfere with interaction between the SARS-CoV-2 spike protein and the ACE-2 receptor used by the virus to invade cells, but the saline does affect the post-infection viral cycle," Guzzo said.

In an earlier article, published in *The Journal of Physical Chemistry Letters*, Guzzo and colleagues showed how interaction between the spike protein and ACE-2 receptor survived different concentrations of NaCl. "The virus probably evolves so as to compensate for fluctuations in ionic strength and maintain an effective medium for cell invasion," she said.

When NaCl molecules enter a cell, the membrane surrounding the cytoplasm is polarized owing to an increase in sodium ions (Na^+). As a result of this energy imbalance, a large amount of the cell's potassium (K^+) is ejected to restore a balance of charges in the membrane (this mechanism is known as the sodium-potassium pump).

Saturation due to the sodium-potassium pump makes the cell expend ATP (adenosine triphosphate), one of the main sources of energy for cellular processes. Consumption of ATP for cellular depolarization prevents the virus from using it to replicate.

"Cells have to get rid of sodium via the sodium-potassium pump, and this uses up their energy store, so there's no ATP left for viral replication," Ulrich explained.

The study also showed that the salt does not affect mitochondrial activity. Mitochondria are dynamic organelles involved in cellular respiration and ATP creation, as well as other metabolic processes. "At these concentrations, the salt doesn't damage the cell. We observed that mitochondria remained healthy throughout the process," Guzzo said.

In the study, the researchers suggest that the use of hypertonic saline could be tested in two ways. One is a nasal spray for prophylaxis of the airways, the main gateway for SARS-CoV-2 to enter the organism. "This type of spray can be found in any pharmacy and could be used prophylactically by front-line health workers or other people who are highly exposed to the virus. If its efficacy is confirmed in clinical trials, it could reduce viral replication in the nose and throat," Guzzo said.

The other strategy they propose is nebulizing the saline into the lungs. In this case, the right concentration of NaCl is essential, and the efficacy of the method can be assessed only in clinical trials involving COVID-19 patients. It is worth recalling that hypertonic saline nebulization is already used to treat children with bronchiolitis, for example.

In the case of respiratory syncytial virus (RSV), the most common cause of bronchiolitis, hypertonic saline is known to reduce infection and inflammation in cultured human respiratory epithelial cells.

"It's not a single solution, and it would have to be used in the first few days after infection," Guzzo said. "Reducing viral replication means reducing the severity of the disease and the inflammatory response. COVID-19 is a complex disease, comprising the viral replication stage, which hypertonic saline could treat, and then systemic inflammation, which is far more extensive. This second stage can be intense and lead to a number of complications in different organs."

More information: Rafael R. G. Machado et al, Inhibition of Severe Acute Respiratory Syndrome Coronavirus 2 Replication by Hypertonic Saline Solution in Lung and Kidney Epithelial Cells, *ACS Pharmacology & Translational Science* (2021). DOI: 10.1021/acsptsci.1c00080

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