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Bicarbonate Effects on Antibacterial Immunity and Mucus Glycobiology in the Cystic Fibrosis Lung: A Review With Selected Experimental Observations

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Abstract

The primary defect in cystic fibrosis (CF) is abnormal chloride and bicarbonate transport in the CF transmembrane conductance regulator epithelial ion channel. The apical surface of the respiratory tract is lined by an airway surface liquid (ASL) layer composed of mucin comprising mainly MUC5A and MUC5B glycoproteins. ASL homeostasis depends on sodium bicarbonate secretion into the airways and secretion deficits alter mucus properties leading to airway obstruction, inflammation and infections. Downstream effects of abnormal ion transport in the lungs include altered intrinsic immune defenses. We observed that neutrophils killed *Pseudomonas aeruginosa* more efficiently when it had been exposed to sodium bicarbonate, and formation of neutrophil extracellular traps by neutrophils was augmented in the presence of increasing bicarbonate concentrations. Physiological levels of bicarbonate sensitized *P. aeruginosa* to the antimicrobial peptide cathelicidin LL-37, which is present in both lung ASL and neutrophil extracellular traps. Sodium bicarbonate has various uses in clinical medicine and in the care of CF patients and could be further explored as a therapeutic adjunct against *Pseudomonas* infections.

Keywords: cystic fibrosis; bicarbonate; mucus; pneumonia; bacterial infection; neutrophil; cathelicidin; neutrophil extracellular traps

Introduction

Cystic fibrosis (CF) is one of the most common life-shortening genetic diseases affecting approximately 31,000 people in the United States and an additional 70,000 people worldwide. While life expectancy continues to rise because of earlier detection and recent treatment advances, the median age of death is calculated to be 34.1 years in the CF Foundation Patient Registry 2020 Annual Data Report.[1] Cystic fibrosis is caused by a mutation in the gene encoding the CF transmembrane conductance regulator (CFTR) and inherited through an autosomal recessive pattern. The CFTR is a transmembrane ATP-binding cassette ion transporter that is required for chloride and bicarbonate secretion on epithelial surfaces, and loss-of-function mutations lead to severe reduction or absence of these anions in the CF lung. More than 2000 *CFTR* mutations have been identified[2] with the most common mutation being deletion of phenylalanine at the 508 position, referred to as Phe508del or ΔF508, which is present in 70% of *CFTR* alleles worldwide.[3] As a multiorgan systemic disease, CF can manifest as chronic sinusitis, chronic respiratory infections, pancreatic exocrine insufficiency, diabetes mellitus, meconium ileus, cirrhosis and infertility due to obstruction of the vas deferens.[4] Vast heterogeneity exists in the clinical presentation and severity of the disease depending on the underlying *CFTR* mutation(s), combination of alleles and other factors such as modifier genes, environment and lifestyle.[5,6]

Cystic fibrosis and altered innate lung defenses

Hallmark characteristics of CF involve a vicious cycle of mucus-obstructed airways, inflammation, and recurrent pulmonary infections. Signs of early lung disease are detected even in young infants with CF. Multiple studies have demonstrated proinflammatory mediators in the CF lung even in the absence of (and before) the development of lung infections.[7,8] However, infection is no doubt a trigger and accelerator of the chronic inflammatory process.[9] The first lower respiratory tract pathogens acquired in CF are typically *Staphylococcus aureus* and *Haemophilus influenzae*. Later on, patients can acquire the gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, which is the most common colonizer of the CF respiratory tract, with 45% of patients having culture-positive respiratory samples. The median age of first infection is 1 year,[10] and once acquired, CF lung infections are almost impossible to eradicate despite aggressive and repeated antibiotic regimens. Antibiotic resistance rates are high and almost a quarter of strains are multidrug-resistant (MDR).[1] *Pseudomonas* infections, in particular, are associated with a decline in lung function and poor outcomes in CF.[11]

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The host immune system mounts a response to bacterial infections by recruiting key effector leukocytes, which provoke a cascade of airway inflammation. Circulating neutrophils are signaled to migrate to the site of infection via microbial pathogen-associated molecular patterns. Once activated, neutrophils generate reactive oxygen species (ROS) and reactive nitrogen species, release proteases and perform phagocytic functions. Stimulated neutrophils can also be activated to release elaborate web-like structures called neutrophil extracellular traps (NETs) through a specialized cell death process termed NETosis. Neutrophil extracellular traps are formed via the extravasation of chromatin structures, that is, nuclear or mitochondrial DNA and histones, into the extracellular space and have the ability to ensnare and kill invading pathogens.[12,13]

Found within NET complexes are antimicrobial peptides (AMPs) that are naturally occurring endogenous defense molecules. Antimicrobial peptides are also expressed in neutrophil granules, macrophages and epithelial cells, including those in the airway—examples include lactoferrin, lysozyme, surfactant B, cationic defensins and cathelicidin LL-37. Antimicrobial peptides have a measure of direct antimicrobial effect on a broad spectrum of microorganisms,[14–16] which has received attention toward clinical translation against antibiotic-resistant strains of *S. aureus* and *P. aeruginosa*.[17,18] These host defense peptides can also have synergistic and additive properties when combined with conventional antibiotics, which may be of particular significance in the current era of expanding drug resistance.[19–21] A prevalent and well-studied AMP, human cathelicidin LL-37, exemplifies the killing activity of this class of defense molecules, specifically targeting the lipid bilayer of bacterial cell membranes to form transmembrane pores leading to bacterial lysis.[22] In addition to their antimicrobial functions, NETs and AMPs also contribute to immune cell differentiation, chemoattraction of neutrophils and monocytes and cytokine release.[23,24] Furthermore, these immune defense factors can play an immunomodulatory role in autoimmune diseases[25–27] and can promote re-epithelialization in wound healing.[28]

Chronic inflammation in CF is driven by an underlying dysfunctional immune response. Neutrophils are activated in this highly inflammatory environment in the airways but are unable to effectively clear bacterial infections due to innate defects in apoptosis, phagocytosis and the generation of ROS.[29–32] Excessive neutrophil degranulation occurs in acidic cytosolic pH levels, leading to the release of primary granules that contain enzymes, such as myeloperoxidase and neutrophil elastase (NE).[33–36] Indeed, NE can serve as a longitudinal biomarker for lung function in CF and is inversely associated with a decline in lung function.[37,38] It has been proposed that imbalanced levels of inflammatory NE proteases cause breakdown of lung parenchyma, leading to lung damage.[39]

Excessive activation of NETosis and the release of NE and extracellular DNA have been associated with ex vivo CF patient neutrophils and in vivo CF pig neutrophils in response to clinical isolates of *P. aeruginosa*.[40–42] Using bicarbonate to increase the pH to an alkaline level also boosted ROS production, histone cleavage, and bacterially induced NETosis. *P. aeruginosa, S. aureus* and the Gram-negative cell wall component lipopolysaccharide contributed to increased NETs in an alkaline pH.[43] Differences in NETs induced by *P. aeruginosa* have been differentiated between "early" bacterial isolates (obtained from CF patients from 3 months to 11 years) and "late" bacterial isolates (obtained 5–20 years later than the earlier sample) with more NETs formed in response to the early isolates.[41] Acquired resistance to NET-mediated killing is associated with late stage *P. aeruginosa* isolates and their mucoid phenotype.[42] Given the early age of acquisition of *P. aeruginosa* and our understanding of NET-killing with late clinical isolates, the CF Foundation recommends the use of recombinant deoxyribonuclease or rDNase, an inhaled enzyme medication that catalyzes the breakdown of DNA, starting at the age of 6 years. Although CF inflammation secondary to neutrophil and related NET formation is well supported in literature, anti-inflammatory treatment options are not routinely used as we have yet to consolidate our experimental findings with the clinical response in human patients.

Mucus and bicarbonate within the airway surface liquid

Airway mucus is composed mainly of water and a much smaller fraction of mucin glycoproteins. There are 21 types of mucins, which are encoded by corresponding *MUC* genes, and they are subcategorized between gel-forming and membrane-bound mucins. Gel-forming mucins MUC5AC and MUC5B are the primary structural components of airway mucus; MUC5B is predominantly expressed in the healthy airway and MUC5AC is upregulated in response to inflammatory conditions including CF.[44] Goblet cells secrete mucus in a process that is dependent on functional CFTR and bicarbonate.[45,46] Treatment of bronchial epithelial cells with the T-helper 2 cytokine interleukin-4 led to goblet cell hyperplasia, increased bicarbonate permeability and secretion, and further augmented release of mucus.[47] Native mucus and isolated mucin glycans dispersed bacterial cells within a mature biofilm into the planktonic state and prevented bacterial attachment to human cells. As such, mucins are innately equipped to regulate bacterial virulence phenotypes.[48] A thin layer of mucus covers the apical (luminal) surface of ciliated respiratory epithelium that comprises the airway surface liquid (ASL). As a major driving factor of airway homeostasis, the ASL regulates ciliary function and mucociliary transport to move entrapped microbes and foreign particles toward the pharynx to be expelled or removed. It also contains innate immune AMPs that contribute to airway defenses.

Normal function of the ASL and airway host defenses depends on bicarbonate transport into the airway lumen.[45–52] Small airways concomitantly secrete and absorb bicarbonate to precisely maintain its steady state concentration within the thin ASL layer.[53] Comparing airway epithelia with CF, non-CF and *CFTR* heterozygous individuals in the piglet model, CFTR expression had a direct relationship to bicarbonate secretion and bacterial killing of *S. aureus*, ASL pH and ASL viscosity.[54] Dysfunctional bicarbonate secretion leads to ASL acidification and changes in ASL volume and ionic concentrations. Increased sodium reabsorption draws water away from the ASL and contributes to the low volume/dehydration hypothesis of CF lung disease.[51] A landmark observation was made by Quinton about the role of bicarbonate secretion on CF "mucoviscidosis." Mucin glycoproteins have large negative repulsive charges but remain in compacted granules through cationic Ca^{2+} interactions. When mucin is released in the presence of normal bicarbonate levels, the bicarbonate anion (HCO_3^-) binds Ca^{2+} and unshields mucin. This exposed electronegative repulsion allows for rapid mucin expansion.[55] In line with these findings, gastrointestinal tract mucus in CF mice was thick and adherent to the epithelium but was restored to normal mucus when secreted into 100 mM sodium bicarbonate buffer.[45] Clinically, thickened and inspissated mucus in the airways causes airway inflammation, resulting in progressive airway disease with structural lung changes. Ultimately, this process drives the significant morbidity and mortality in the CF population.[55–57]

Bicarbonate is a robust buffer that maintains a tightly regulated physiologic pH in the blood. The renal system reabsorbs sodium

bicarbonate when the blood acid-base system becomes acidotic. A pH value of 7.40 is ideal for aerobic cellular respiration and other biochemical processes. Physiologic concentrations of bicarbonate vary depending on the organ and designated function. The pancreas is a major bicarbonate secretor, and the pancreatic ductular fluid concentration is maintained at 150 mM. Bicarbonate levels in the blood and salivary glands are 24 mM and 60 mM, respectively. In vivo measurements of ASL bicarbonate concentrations have varied widely because of technical challenges, such as effects of the measuring electrode on transport equilibrium and CO_2 variations during breathing,[58,59] but there is a general consensus that pH is more alkaline in the lower airways (eg, bronchi) than in the upper airways (eg, nasal mucosa).[58,60] The ASL pH has also been estimated in vitro using monolayers of the human bronchial adenocarcinoma cell line Calu3, which exhibit cyclic adenosine monophosphate (cAMP)–stimulated, CFTR-dependent bicarbonate transport. Calu3 model estimates of ASL bicarbonate concentrations have ranged from 10–20 mM[61] to 25–30 mM,[62,63] likely reflecting a measure of dynamic responsiveness to experimental conditions. However, from both in vivo and in vitro experimental studies, the pH and bicarbonate buffering capacity calculated in normal respiratory ASL (CFTR function intact) has always been higher than in CF ASL.[64–66] For example, one study reported a normal ASL pH value of 7.18 and a CF ASL pH value of 6.57,[64] and another investigation calculated the bicarbonate buffering capacity of ASL from CFTR-deficient Calu3 monolayers dropping by more than 50% compared with that from normal Calu3 cells.[67]

Bicarbonate administration in clinical medicine

In patient care settings, sodium bicarbonate is given intravenously in cases of severe sepsis or metabolic acidosis to raise the blood pH if it becomes exceedingly acidotic.[68,69] Treatment with sodium bicarbonate in patients with chronic kidney disease and chronic metabolic acidosis delays further progression of disease and prevents contrast-induced kidney disease when undergoing contrast procedures.[70,71] Sodium bicarbonate is an antidote for cardiac arrhythmias caused by antidepressants and other sodium channel blocker medications.[72] Bicarbonate also reverses toxic ingestions of salicylate and methanol ingestion.[73] There have been studies on improving performance levels of severe intensity exercises with sodium bicarbonate supplementation. The use of sodium bicarbonate as an antibacterial agent was initially described against periodontal pathogens[74,75] and has been implicated for dental hygiene use.[76] The varied interest in the use of bicarbonate can be attributed to being widely accessible, having a favorable adverse effect profile, low toxicity and low cost.

Inhaled bicarbonate use has been safe and tolerated as a therapeutic agent in the care of CF patients. The anion can be given via inhalation as a mucolytic in CF and possibly in other mucus-obstructed airway diseases, such as chronic obstructive pulmonary disease and non-CF bronchiectasis. A small prospective clinical study was recently published on the use of twice daily inhaled sodium bicarbonate. There were no adverse events associated with taking the aerosolized agent. After a 10-week administration period, sputum pH increased as well as sputum rheology moduli (elasticity, viscosity and viscoelasticity), and this correlated favorably to lung function and quality of life.[77] Other clinical trials using inhaled sodium bicarbonate in CF patients are in progress. These studies are measuring the effect of sodium bicarbonate on mucociliary clearance, sputum pH and pretreatment and posttreatment pulmonary function tests.[78,79] Successful case study reports of inhaled sodium bicarbonate in COVID-19 have also prompted clinical trials investigating this urgent area of study.[80,81]

Direct and indirect antimicrobial effects of bicarbonate

Sodium bicarbonate can function as an antimicrobial agent either alone[82] or synergistically with other antimicrobials.[83,84] Bicarbonate was first shown to inhibit the growth of various aerobic and anaerobic microorganisms, such as *Escherichia coli*, *Pseudomonas* species, *Lactobacillus plantarum* and *Saccharomyces* yeast species and later extended to *S. aureus*.[82,83] Subsequent studies validated its effectiveness in artificial sputum media that more closely mimics physiologic conditions.[85] Aminoglycosides demonstrated synergistic growth inhibition of *E. coli* in the presence of bicarbonate.[83] Tobramycin, an aminoglycoside commonly used in CF clinical care, synergistically killed *P. aeruginosa* in the planktonic form and further antagonized biofilm formation.[84]

The transition from planktonic bacteria to biofilms is a sign of chronic colonization in the CF respiratory airways. Early bacterial aggregates stimulate neutrophil activation but are able to evade neutrophil killing and continue to form biofilms.[86–88] Mature biofilms self-produce extracellular matrix components, such as alginate, polysaccharide synthesis locus and pellicle that protect them from host defenses. The biofilm bacteria become tolerant and eventually resistant to antibiotics. Ongoing inflammation and recurrent infections lead to challenges in diagnosis and clinical treatment of patients.[89,90] Interestingly, *P. aeruginosa* biofilm formation can be impeded in 100 mM sodium bicarbonate via increased production of intracellular cAMP production in *P. aeruginosa,* independent of osmolality or the ionic strength of the solution.[91]

Selected experimentation

Performing studies that mimic CF airways remains challenging. Greater knowledge regarding the antimicrobial and innate immune system response to bicarbonate in the context of CF pathogens and their interactions with neutrophils inspired the short, focused set of research studies hereinafter.

Bacterial strains and growth conditions

Pseudomonas aeruginosa strains used include the widely studied reference strain PAO1 initially isolated from a human wound, P4, an MDR human clinical lung isolate obtained from a tertiary academic hospital in the New York metropolitan area, and two clinical strains[92,93] cultured from the sputum of CF patients at Rady Children's Hospital San Diego. Bacterial strains were routinely streaked from glycerol stocks onto Luria Broth (LB) plates biweekly and overnight cultures in LB were diluted 1:10 in LB ±25 mM sodium bicarbonate and incubated at 37°C with shaking to mid-log phase (OD_{600} = 0.4).

Ethics statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments. Under a UC San Diego approved institutional review board protocol (approval #131002, annual approval latest June 15, 2022) with informed consent, healthy adult human donors provided venous blood collected with heparin as an anticoagulant.

Pseudomonas aeruginosa exposed to bicarbonate are more efficiently killed by neutrophils

Neutrophils were isolated from human blood with PolymorphPrep (Axis-Shield) per manufacturer's instructions, diluted to 1×10^7 cells/mL in Hank's balanced salt solution, and seeded into a 2-mL Eppendorf tube at 3×10^6 cells/mL. Sodium bicarbonate buffers of varying concentration were prepared from a 1M stock solution (Sigma-Aldrich, Cat # S6014) in Roswell Park Memorial Institute medium (RPMI, Gibco, Cat #11835030) + 10% LB added to Eppendorf tubes. PAO1 bacteria were added at a multiplicity of infection of 0.01 bacteria/neutrophil, tubes were rotated at 37°C for 1 hour, and then neutrophils were lysed by sonication and dilution plated on LB to enumerate bacterial colony-forming units (CFU). *Pseudomonas aeruginosa* grown in physiologic bicarbonate (25 mM) were more efficiently killed by human neutrophils compared with bacteria grown in the absence of bicarbonate (Figure 1A).

Bicarbonate enhances NET formation

Airway surface liquid is the site of complex ionic interactions that are perturbed in CF. We previously showed that NETosis was reduced in the absence of extracellular chloride, the first deficient anion discovered in CF.[94] To study the effect of bicarbonate on NET formation, we seeded human neutrophils at 2×10^5 cells/well onto 48-well plates with sodium bicarbonate at 4 mM (buffer alone), 10 mM (low) and 24 mM (physiologic) concentrations. Phorbol 12-myristate 13-acetate (PMA, 25 nM) was added to stimulate NET formation at 37°C + 5% CO_2 for 3 hours. After this, micrococcal nuclease was added at 500 mU to digest extracellular DNA, and the reaction was stopped with 5 mM ethylenediaminetetraacetic acid. Samples were centrifuged at 200*g* for 8 minutes. The supernatant was transferred to a separate 96-well flat bottom plate, and extracellular DNA was quantified using the Quant-iT PicoGreen assay kit (Life Technologies). As shown in Figure 1B, we observed a significant difference in the production of NETs between the lowest concentration at 4 mM and both the 10 mM and 24 mM concentrations ($P = 0.04$ and 0.001, respectively); more NETs were produced at 24 mM compared with 10 mM ($P = 0.004$).

Neutrophil extracellular traps were visualized per a protocol previously described by von Köckritz-Blickwede et al.[95,96] Briefly, slides were fixed by adding paraformaldehyde at a final concentration of 4%. Slides were blocked by adding 2% bovine serum albumin–phosphate buffered saline + 2% goat serum. Slides were washed with phosphate buffered saline 3 times between each step. Rabbit antihuman myeloperoxidase (Dako #A0398) was added, and after 1-hour incubation, the second antibody AlexaFluor488 goat anti-rabbit immunoglobulin G (IgG) (Invitrogen #A11070) was added. Slides were then embedded in ProlongGold antifade + DAPI (Invitrogen #P36931). Samples were examined using an inverted confocal laser-scanning 2-photon Olympus Fluoview FV1000 microscope with Fluoview Spectral Scanning Technology (Olympus), and representative images are shown in Figure 1C.

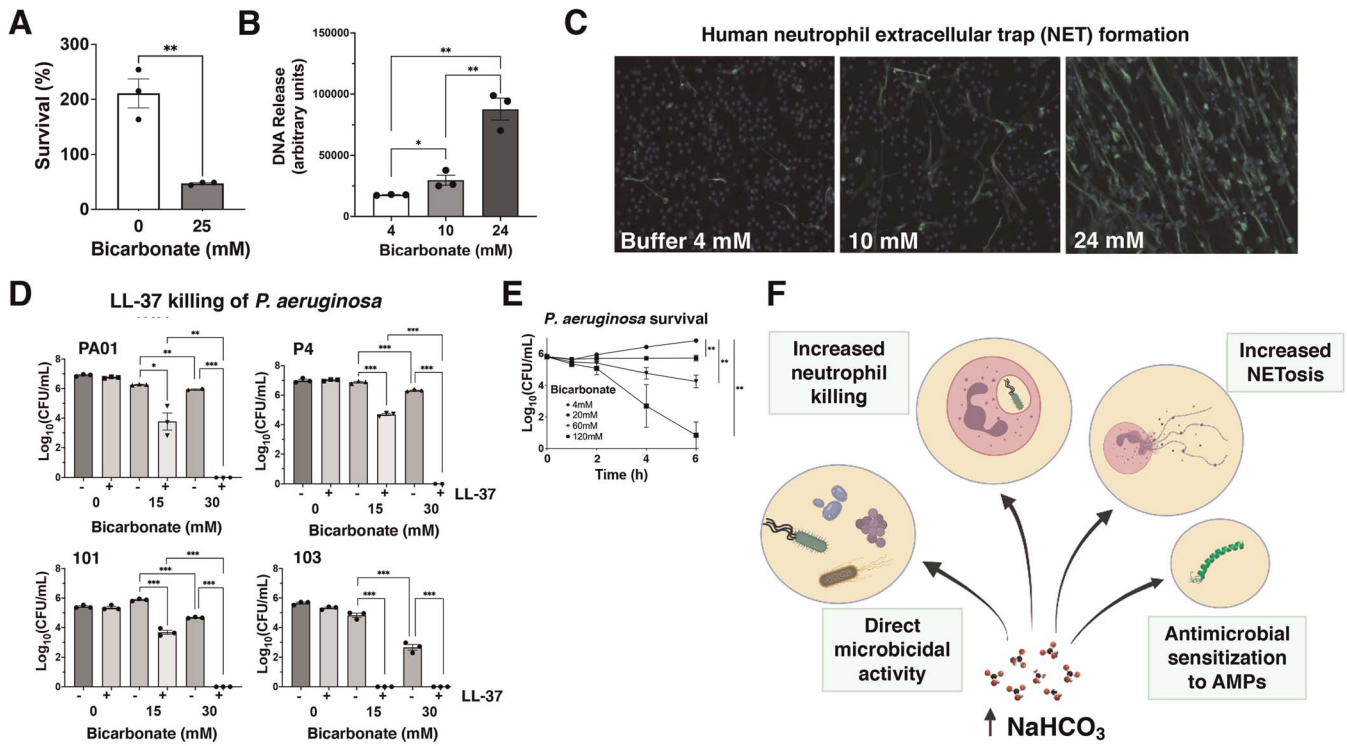

Figure 1. Effects of bicarbonate concentration on *Pseudomonas aeruginosa* innate immune susceptibility and virulence. A: Neutrophil killing of *P. aeruginosa* strain P4 grown in the presence or absence of 25 mM bicarbonate in the overnight culture. B: PicoGreen quantification of PMA-induced human NET DNA release in different bicarbonate concentrations. C: Representative images of PMA-induced NETs in different bicarbonate concentrations by antihuman myeloperoxidase antibody and AlexaFluor488-conjugated secondary antibody. D: Survival of *P. aeruginosa* strains in the presence (+) or absence (–) of 4 μM LL-37 at the indicated bicarbonate concentrations. E: Killing kinetics of *P. aeruginosa* in increasing concentrations of bicarbonate. F: Schematic summary of the effects of bicarbonate on *P. aeruginosa* innate immune susceptibility and virulence. Unpaired parametric *t* tests (A, B) and 2-way analysis of variance (D, E) were performed; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$. AMPs, antimicrobial peptides; CFU, colony-forming unit; NET, neutrophil extracellular trap; PMA, phorbol 12-myristate 13-acetate.

Sodium bicarbonate enhances the intrinsic activity of innate AMP LL-37

LL-37 facilitates the formation and stabilization of NETs,[97] and an optimal ionic environment is crucial for AMP activity, as pH buffering changes can influence their native cationic charge and killing properties.[98,99] We performed kinetic killing curves for LL-37 against *P. aeruginosa* strains over a range of bicarbonate concentrations, using bicarbonate concentrations of 30 mM as a representative of bicarbonate concentration in the normal lung and 15 mM as a reduced concentration approximating a secretion defect as might be found in the CF lung. Log phase bacteria at OD_{600} = 0.4 were washed twice and diluted with RPMI with 10% LB to an initial inoculum of 2×10^6 CFU/mL. LL-37 (Bachem, Cat #4042456) and a 1M sodium bicarbonate stock solution were diluted in the same media to the desired concentrations as indicated. Assays were conducted in a flat 96-well plate with a total volume of 200 µL, and each condition was tested in triplicate. Plates were placed into a 37°C incubator with shaking and removed after 4 hours. Aliquots were taken from the plate and serially diluted for CFU enumeration. We found that AMP activity of LL-37 could be augmented with sodium bicarbonate and was greater at higher (physiologic) concentrations versus lower (CF) concentrations (Figure 1D).

Direct antimicrobial effect of bicarbonate on P. aeruginosa

Airway cells cultured from CF patients possess inherent defects in AMP activity compared with cells from non-CF patients, and aerosolizing high-dose sodium bicarbonate (100 mM) into the airway ASL of CF pigs enhanced killing of *S. aureus*–coated grids.[66] Prior reports of direct antimicrobial activity of bicarbonate against *P. aeruginosa* tested concentrations of 100 mM or greater.[85,91] Using the MDR human lung clinical *P. aeruginosa* isolate P4, we found dose-dependent reduction in *P. aeruginosa* CFUs occurred at bicarbonate concentrations of 60 or 120 mM, suggesting the potential of the aerosolized agent to exert direct antimicrobial activity against the pathogen (Figure 1E).

Statistical analyses

Statistical analyses were performed using GraphPad Prism, v8.1.2 (GraphPad Software, Inc, La Jolla, Calif). Unpaired parametric *t* tests and 2-way analysis of variance were performed, and a *P* value less than 0.05 was considered statistically significant.

Conclusions and areas for future study

A defect in bicarbonate secretion into the airways in CF causes pathophysiologic changes in mucus biology, the ASL, and components within the ASL. Here, we have outlined our understanding of sodium bicarbonate as an antimicrobial agent against various microorganisms, and specifically MDR and CF clinical isolates of *P. aeruginosa*, and bicarbonate in concert with ASL antimicrobial peptides including cathelicidin LL-37. The antimicrobial effect seen at higher concentrations of bicarbonate is not due to osmolality or ionic strength.[91] This suggests that increased concentrations may contribute as a defense mechanism against bacterial infection and chronic colonization in the lungs. Obstructive CF sputum consists of extracellular DNA,[100–102] and clinical treatment targets the breakdown of NETs. We present a novel perspective on how alterations in sodium bicarbonate affect the innate immune system. We found that sodium bicarbonate stimulates NETosis, and pretreating *P. aeruginosa* with sodium bicarbonate enhanced bacterial killing effects of neutrophils. Sodium bicarbonate appears to increase bacterial susceptibility to killing through increasing intracellular bacterial cAMP levels and disrupting the pH gradient of the proton motive force across the cytoplasmic membrane of gram-negative and gram-positive bacteria.[91,92] It is possible that commensal lung bacteria may also be impacted with augmented levels of bicarbonate. The extent of such effects likely depends on the condition of the lung, because the physiologic level of bicarbonate is dynamic in nature. Introduction of bacteria due to an acute infection may result in a precipitous decrease in bicarbonate levels. Treatment with sodium bicarbonate can theoretically raise bicarbonate to levels those pathogenic bacteria are not accustomed to and, thus perhaps more susceptible to, in comparison with commensal bacteria of the upper respiratory tract. The multifaceted potential contributions of bicarbonate to airway host defense are summarized schematically in Figure 1F.

Given the previous considerations, the use of inhaled bicarbonate continues to be investigated as a potential therapeutic in CF and other respiratory diseases. The development of CFTR modulators within the last decade has created landmark shifts in the treatment and outcome of patients with CF. These treatments have led to increased lung function, weight gain, reduction of pulmonary exacerbations and improvement in quality of life. Gene-directed therapies are currently available for 82%–90% of CF patients worldwide, as a result of the inclusion of one copy of DF508 as a qualifying mutation.[103] These small molecules target *CFTR* mutations, alter the malfunctioning protein, and restore functional expression. Drugs are classified based on their effect on the gene and are either termed "potentiators," which increase the gating of the CFTR chloride-bicarbonate ion channel at the cell surface, or "correctors," which act to rescue posttranslational folding, processing, and trafficking of the protein. In the presence of CFTR modulating drugs, restored chloride conductance can result in normalization of sweat chloride levels. Bicarbonate transport activity was rescued in cells expressing p.F508del-CFTR. By calculating HCO_3^- influx from the pH, a HCO_3^- influx of 17–25.8 mM occurred in the presence of one corrector and 45.9 mM with two correctors.[93] Because CFTR modulators treat the underlying defect, the downstream effects of an abnormal CFTR are mitigated. For 10%–18% of patients or more in countries where the prevalence of ΔF508 is higher and who do not have therapeutic options, the use of sodium bicarbonate as a therapeutic may be more relevant. The safety and tolerability profile of bicarbonate make it an agreeable medication to be considered for long-term use. As such, its therapeutic application can be considered a step toward restoring the depressed bicarbonate levels characteristic of CF airways. Long-term inhaled bicarbonate therapy may create an environment in which colonized *P. aeruginosa* is constantly exposed to sodium bicarbonate, which renders it more sensitized to the defenses of the immune system. There likely also exists a synergistic effect of pretreated bacteria with additional host innate immune factors that can be further investigated.

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References

[1] Cystic Fibrosis Foundation. *Patient Registry 2020 Annual Data Report*. Available at: https://wwwcfforg/sites/default/files/2021-11/Patient-Registry-Annual-Data-Reportpdf Published November 2021. Accessed May 20, 2022.

[2] Cystic Fibrosis Mutation Database. Available at: http://www.genet.sickkids.on.ca/. Accessed July 7, 2021.
[3] Gentzsch M, Mall MA. Ion channel modulators in cystic fibrosis. *Chest* 2018;154(2):383–393. doi: 10.1016/j.chest.2018.04.036
[4] Kunzelmann K, Schreiber R, Hadorn HB. Bicarbonate in cystic fibrosis. *J Cystic Fibros* 2017;16(6):653–662. doi: 10.1016/j.jcf.2017.06.005
[5] Li W, Soave D, Miller MR, et al. Unraveling the complex genetic model for cystic fibrosis: pleiotropic effects of modifier genes on early cystic fibrosis-related morbidities. *Hum Genet* 2014;133(2):151–161. doi: 10.1007/s00439-013-1363-7
[6] Corvol H, Blackman SM, Boëlle PY, et al. Genome-wide association meta-analysis identifies five modifier loci of lung disease severity in cystic fibrosis. *Nat Commun* 2015;6:8382. doi: 10.1038/ncomms9382
[7] Tirouvanziam R, Khazaal I, Péault B. Primary inflammation in human cystic fibrosis small airways. *Am J Physiol Lung Cell Mol Physiol* 2002;283(2):L445–L451. doi: 10.1152/ajplung.00419.2001
[8] Fritzsching B, Zhou-Suckow Z, Trojanek JB, et al. Hypoxic epithelial necrosis triggers neutrophilic inflammation via IL-1 receptor signaling in cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2015;191(8):902–913. doi: 10.1164/rccm.201409-1610OC
[9] Armstrong DS, Hook SM, Jamsen KM, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. *Pediatr Pulmonol* 2005;40(6):500–510. doi: 10.1002/ppul.20294
[10] Li Z, Kosorok MR, Farrell PM, et al. Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis. *JAMA* 2005;293(5):581–588. doi: 10.1001/jama.293.5.581
[11] Langton Hewer SC, Smyth AR. Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. *Cochrane Database Syst Rev* 2017;4(4):CD004197. doi: 10.1002/14651858.CD004197.pub5
[12] Brinkmann V. Neutrophil extracellular traps in the second decade. *J Innate Immun* 2018;10(5–6):414–421. doi: 10.1159/000489829
[13] Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;303(5663):1532–1535. doi: 10.1126/science.1092385
[14] Nizet V, Ohtake T, Lauth X, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 2001;414(6862):454–457. doi: 10.1038/35106587
[15] Nizet V. Antimicrobial peptide resistance mechanisms of human bacterial pathogens. *Curr Issues Mol Biol* 2006;8(1):11–26. doi: 10.21775/cimb.008.011
[16] Chromek M, Slamová Z, Bergman P, et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat Med* 2006;12(6):636–641. doi: 10.1038/nm1407
[17] Shurko JF, Galega RS, Li C, Lee GC. Evaluation of LL-37 antimicrobial peptide derivatives alone and in combination with vancomycin against *S. aureus*. *J Antibiot* 2001;414(6862):454–457. doi: 10.1038/s41429-018-0090-7
[18] Dosler S, Karaaslan E. Inhibition and destruction of *Pseudomonas aeruginosa* biofilms by antibiotics and antimicrobial peptides. *Peptides* 2014;62:32–37. doi: 10.1016/j.peptides.2014.09.021
[19] Zucca M, Savoia D. The post-antibiotic era: promising developments in the therapy of infectious diseases. *Int J Biomed Sci* 2010;6(2):77–86.
[20] Kumaraswamy M, Lin L, Olson J, et al. Standard susceptibility testing overlooks potent azithromycin activity and cationic peptide synergy against MDR *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 2016;71(5):1264–1269. doi: 10.1093/jac/dkv487
[21] Lin L, Nonejuie P, Munguia J, et al. Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant gram-negative bacterial pathogens. *EBioMedicine* 2015;2(7):690–698. doi: 10.1016/j.ebiom.2015.05.021
[22] Lee CC, Sun Y, Qian S, Huang HW. Transmembrane pores formed by human antimicrobial peptide LL-37. *Biophys J* 2011;100(7):1688–1696. doi: 10.1016/j.bpj.2011.02.018
[23] Steinstraesser L, Kraneburg U, Jacobsen F, Al-Benna S. Host defense peptides and their antimicrobial-immunomodulatory duality. *Immunobiology* 2011;216(3):322–333. doi: 10.1016/j.imbio.2010.07.003
[24] Yang D, Chen Q, Schmidt AP, et al. LLl-37, the neutrophil granule–and epithelial cell–derived cathelicidin, utilizes formyl peptide receptor–like 1 (Fprl1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* 2000;192(7):1069–1074. doi: 10.1084/jem.192.7.1069
[25] Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. *J Immunol* 2013;191(10):4895–4901. doi: 10.4049/jimmunol.1302005
[26] Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med* 2011;3(73):73ra19. doi: 10.1126/scitranslmed.3001180
[27] Lande R, Gregorio J, Facchinetti V, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 2007;449(7162):564–569. doi: 10.1038/nature06116
[28] Tokumaru S, Sayama K, Shirakata Y, et al. Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37. *J Immunol* 2005;175(7):4662–4668. doi: 10.4049/jimmunol.175.7.4662
[29] Painter RG, Valentine VG, Lanson NA Jr, et al. CFTR expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. *Biochemistry* 2006;45(34):10260–10269. doi: 10.1021/bi060490t
[30] Bonfield TL, Hodges CA, Cotton CU, Drumm ML. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. *J Leukoc Biol* 2012;92(5):1111–1122. doi: 10.1189/jlb.0412188
[31] Gray RD, Hardisty G, Regan KH, et al. Delayed neutrophil apoptosis enhances NET formation in cystic fibrosis. *Thorax* 2018;73(2):134–144. doi: 10.1136/thoraxjnl-2017-210134
[32] Moriceau S, Lenoir G, Witko-Sarsat V. In cystic fibrosis homozygotes and heterozygotes, neutrophil apoptosis is delayed and modulated by diamide or roscovitine: evidence for an innate neutrophil disturbance. *J Innate Immun* 2010;2(3):260–266. doi: 10.1159/000295791
[33] Stockley RA. Neutrophils and protease/antiprotease imbalance. *Am J Respir Crit Care Med* 1999;160(5 Pt 2):S49–S52. doi: 10.1164/ajrccm.160.supplement_1.13
[34] Koller DY, Urbanek R, Götz M. Increased degranulation of eosinophil and neutrophil granulocytes in cystic fibrosis. *Am J Respir Crit Care Med* 1995;152(2):629–633. doi: 10.1164/ajrccm.152.2.7633718
[35] Taggart C, Coakley RJ, Greally P, Canny G, O'Neill SJ, McElvaney NG. Increased elastase release by CF neutrophils is mediated by tumor necrosis factor-α and interleukin-8. *Am J Physio Lung Cell Mol Physiol* 2000;278(1):L33–L41. doi: 10.1152/ajplung.2000.278.1.L33
[36] Pohl K, Hayes E, Keenan J, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. *Blood* 2014;124(7):999–1009. doi: 10.1182/blood-2014-02-555268
[37] Mayer-Hamblett N, Aitken ML, Accurso FJ, et al. Association between pulmonary function and sputum biomarkers in cystic fibrosis. *Am J Respir Crit Care Med* 2007;175(8):822–828. doi: 10.1164/rccm.200609-1354OC
[38] Sagel SD, Sontag MK, Wagener JS, Kapsner RK, Osberg I, Accurso FJ. Induced sputum inflammatory measures correlate with lung function in children with cystic fibrosis. *J Pediatr* 2002;141(6):811–817. doi: 10.1067/mpd.2002.129847
[39] Margaroli C, Garratt LW, Horati H, et al. Elastase exocytosis by airway neutrophils Is associated with early lung damage in children with cystic fibrosis. *Am J Respir Crit Care Med* 2019;199(7):873–881. doi: 10.1164/rccm.201803-0442OC
[40] Law SM, Gray RD. Neutrophil extracellular traps and the dysfunctional innate immune response of cystic fibrosis lung disease: a review. *J Inflamm* 2017;14:29. doi: 10.1186/s12950-017-0176-1
[41] Yoo DG, Floyd M, Winn M, Moskowitz SM, Rada B. NET formation induced by *Pseudomonas aeruginosa* cystic fibrosis isolates measured as release of myeloperoxidase–DNA and neutrophil elastase–DNA complexes. *Immunol Lett* 2014;160(2):186–194. doi: 10.1016/j.imlet.2014.03.003
[42] Young RL, Malcolm KC, Kret JE, et al. Neutrophil extracellular trap (NET)–mediated killing of *Pseudomonas aeruginosa*: evidence of acquired resistance within the CF airway, independent of CFTR. *PLoS One* 2011;6(9):e23637. doi: 10.1371/journal.pone.0023637
[43] Khan MA, Philip LM, Cheung G, et al. Regulating NETosis: increasing pH promotes NADPH oxidase-dependent NETosis. *Front Med* 2018;5:19. doi: 10.3389/fmed.2018.00019
[44] Ma J, Rubin BK, Voynow JA. Mucins, mucus, and goblet cells. *Chest* 2018;154(1):169–176. doi: 10.1016/j.chest.2017.11.008
[45] Gustafsson JK, Ermund A, Ambort D, et al. Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. *J Exp Med* 2012;209(7):1263–1272. doi: 10.1084/jem.20120562

[46] Garcia MA, Yang N, Quinton PM. Normal mouse intestinal mucus release requires cystic fibrosis transmembrane regulator-dependent bicarbonate secretion. *J Clin Invest* 2009;119(9):2613–2622. doi: 10.1172/JCI38662
[47] Gorrieri G, Scudieri P, Caci E, et al. Goblet cell hyperplasia requires high bicarbonate transport to support mucin release. *Sci Rep* 2016;6:36016. doi: 10.1038/srep36016
[48] Wheeler KM, Cárcamo-Oyarce G, Turner BS, et al. Mucin glycans attenuate the virulence of *Pseudomonas aeruginosa* in infection. *Nat Microbiol* 2019;4(12):2146–2154. doi: 10.1038/s41564-019-0581-8
[49] Joo NS, Krouse ME, Wu JV, et al. HCO_3^- transport in relation to mucus secretion from submucosal glands. *JOP* 2001;2(4 suppl):280–284.
[50] Ballard ST, Trout L, Mehta A, Inglis SK. Liquid secretion inhibitors reduce mucociliary transport in glandular airways. *Am J Physiol Lung Cell Mol Physiol* 2002;283(2):L329–L335. doi: 10.1152/ajplung.00277.2001
[51] Trout L, King M, Feng W, Inglis SK, Ballard ST. Inhibition of airway liquid secretion and its effect on the physical properties of airway mucus. *Am J Physiol* 1998;274(2):L258–L263. doi: 10.1152/ajplung.1998.274.2.L258
[52] Cooper JL, Quinton PM, Ballard ST. Mucociliary transport in porcine trachea: differential effects of inhibiting chloride and bicarbonate secretion. *Am J Physiol Lung Cell Mol Physiol* 2013;230(3):L184–L190. doi: 10.1152/ajplung.00143.2012
[53] Shamsuddin AKM, Quinton PM. Concurrent absorption and secretion of airway surface liquids and bicarbonate secretion in human bronchioles. *Am J Physiol Lung Cell Mol Physiol* 2019;316(5):L953–L960. doi: 10.1152/ajplung.00545.2018
[54] Shah VS, Ernst S, Tang XX, et al. Relationships among CFTR expression, HCO_3^- secretion, and host defense may inform gene- and cell-based cystic fibrosis therapies. *Proc Natl Acad Sci U S A* 2016;113(19):5382–5387. doi: 10.1073/pnas.1604905113
[55] Quinton PM. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. *Lancet* 2008;372(9636):415–417. doi: 10.1016/S0140-6736(08)61162-9
[56] Chen EY, Yang N, Quinton PM, Chin WC. A new role for bicarbonate in mucus formation. *Am J Physiol Lung Cell Mol Physiol* 2010;299:542–549. doi: 10.1152/ajplung.00180.2010
[57] Quinton PM. The neglected ion: HCO_3^-. *Nat Med* 2001;7(3):292–293. doi: 10.1038/85429
[58] Zajac M, Dreano E, Edwards A, Planelles G, Sermet-Gaudelus I. Airway surface liquid pH regulation in airway epithelium current understandings and gaps in knowledge. *Int J Mol Sci* 2021;22(7):3384. doi: 10.3390/ijms22073384
[59] Benedetto R, Centeio R, Ousingsawat J, Schreiber R, Janda M, Kunzelmann K. Transport properties in $CFTR^{-/-}$ knockout piglets suggest normal airway surface liquid pH and enhanced amiloride-sensitive Na^+ absorption. *Pflugers Arch* 2020;472(10):1507–1519. doi: 10.1007/s00424-020-02440-y
[60] McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EW. Airway surface pH in subjects with cystic fibrosis. *Eur Respir J* 2003;21(1):37–42. doi: 10.1183/09031936.03.00027603
[61] Borowitz D. CFTR, bicarbonate, and the pathophysiology of cystic fibrosis. *Pediatr Pulmonol* 2015;50(S40):S24–S30. doi: 10.1002/ppul.23247
[62] Shan J, Liao J, Huang J, et al. Bicarbonate-dependent chloride transport drives fluid secretion by the human airway epithelial cell line Calu-3. *J Physiol* 2012;590(21):5273–5297. doi: 10.1113/jphysiol.2012.236893
[63] Garnett JP, Hickman E, Burrows R, et al. Novel role for pendrin in orchestrating bicarbonate secretion in cystic fibrosis transmembrane conductance regulator (CFTR)–expressing airway serous cells. *J Biol Chem* 2011;286(47):41069–41082. doi: 10.1074/jbc.M111.266734
[64] Song Y, Salinas D, Nielson DW, Verkman AS. Hyperacidity of secreted fluid from submucosal glands in early cystic fibrosis. *Am J Physiol Cell Physiol* 2006;290(3):C741–C749. doi: 10.1152/ajpcell.00379.2005
[65] Li X, Tang XX, Vargas Buonfiglio LG, et al. Electrolyte transport properties in distal small airways from cystic fibrosis pigs with implications for host defense. *Am J Physiol Lung Cell Mol Physiol* 2016;310(7):L670–L679. doi: 10.1152/ajplung.00422.2015
[66] Pezzulo AA, Tang XX, Hoegger MJ, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature* 2012;487(7405):109–113. doi: 10.1038/nature11130
[67] Kim D, Liao J, Hanrahan JW. The buffer capacity of airway epithelial secretions. *Front Physiol* 2014;5:188. doi: 10.3389/fphys.2014.00188
[68] Jaber S, Paugam C, Futier E, et al. Sodium bicarbonate therapy for patients with severe metabolic acidaemia in the intensive care unit (BICAR-ICU): a multicentre, open-label, randomised controlled, phase 3 trial. *Lancet* 2018;392(10141):31–40. doi: 10.1016/S0140-6736(18)31080-8
[69] Adeva-Andany MM, Fernández-Fernández C, Mouriño-Bayolo D, Castro-Quintela E, Domínguez-Montero A. Sodium bicarbonate therapy in patients with metabolic acidosis. *ScientificWorldJournal* 2014, 2014;627673. doi: 10.1155/2014/627673
[70] Di Iorio BR, Bellasi A, Raphael KL, et al. Treatment of metabolic acidosis with sodium bicarbonate delays progression of chronic kidney disease: the UBI Study. *J Nephrol* 2019;32(6):989–1001. doi: 10.1007/s40620-019-00656-5
[71] Zhang B, Liang L, Chen W, Liang C, Zhang S. The efficacy of sodium bicarbonate in preventing contrast-induced nephropathy in patients with pre-existing renal insufficiency: a meta-analysis. *BMJ Open* 2015;5(3):e006989. doi: 10.1136/bmjopen-2014-006989
[72] Bruccoleri RE, Burns MM. A literature review of the use of sodium bicarbonate for the treatment of QRS widening. *J Med Toxicol* 2016;12(1):121–129. doi: 10.1007/s13181-015-0483-y
[73] Mirrakhimov AE, Ayach T, Barbaryan A, Talari G, Chadha R, Gray A. The role of sodium bicarbonate in the management of some toxic ingestions. *Int J Nephrol* 2017;2017:7831358. doi: 10.1155/2017/7831358
[74] Newbrun E, Hoover CI, Ryder MI. Bactericidal action of bicarbonate ion on selected periodontal pathogenic microorganisms. *J Periodontol* 1984;55(11):658–667. doi: 10.1902/jop.1984.55.11.658
[75] Cerra MB, Killoy WJ. The effect of sodium bicarbonate and hydrogen peroxide on the microbial flora of periodontal pockets. A preliminary report. *J Periodontol* 1982;53(10):599–603. doi: 10.1902/jop.1982.53.10.599
[76] Drake D. Antibacterial activity of baking soda. *Compend Contin Educ Dent Suppl* 1997;18(21):S17–S21.
[77] Gomez CCS, Parazzi PLF, Clinckspoor KJ, et al. Safety, tolerability, and effects of sodium bicarbonate inhalation in cystic fibrosis. *Clin Drug Investig* 2020;40(2):105–117. doi: 10.1007/s40261-019-00861-x
[78] ClinicalTrials.gov. Effects of inhaled bicarbonate on airway pH in cystic fibrosis. Available at: https://clinicaltrials.gov/ct2/show/NCT03391414. Updated January 5, 2018. Accessed September 6, 2021.
[79] ClinicalTrials.gov. Inhaled bicarbonate therapy in cystic fibrosis. Available at: https://clinicaltrials.gov/ct2/show/NCT00177645. Updated February 17, 2016. Accessed September 6, 2021.
[80] Wardeh A, Conklin J, Ko M. Case reports of observed significant improvement in patients with ARDS due to COVID-19 and maximum ventilatory support after inhalation of sodium bicarbonate. *J Clin Intensive Care Med* 2020;5:16–19. doi: 10.29328/journal.jcicm.1001029
[81] ClinicalTrials.gov. The role of sodium bicarbonate as an adjuvant treatment of computed tomography identified COVID-19 pneumonia. Available at: https://clinicaltrials.gov/ct2/show/NCT04374591. Updated: October 6, 2020. Accessed September 6, 2021.
[82] Corral LG, Post LS, Montville TJ. Antimicrobial activity of sodium bicarbonate: a research note. *J Food Sci* 1988;53(3):981–982. doi: 10.1111/j.1365-2621.1988.tb09005.x
[83] Gutiérrez-Huante M, Martínez H, Bustamante VH, Puente JL, Sánchez J. Bicarbonate enhances the in vitro antibiotic activity of kanamycin in Escherichia coli. *Lett Appl Microbiol* 2015;60(5):440–446. doi: 10.1111/lam.12388
[84] Kaushik KS, Stolhandske J, Shindell O, Smyth HD, Gordon VD. Tobramycin and bicarbonate synergise to kill planktonic *Pseudomonas aeruginosa*, but antagonise to promote biofilm survival. *NPJ Biofilms Microbiomes* 2016;2:16006. doi: 10.1038/npjbiofilms.2016.6
[85] Jaikumpun P, Ruksakiet K, Stercz B, et al. Antibacterial effects of bicarbonate in media modified to mimic cystic fibrosis sputum. *Int J Mol Sci* 2020;21(22):8614. doi: 10.3390/ijms21228614
[86] Pestrak MJ, Chaney SB, Eggleston HC, et al. *Pseudomonas aeruginosa* rugose small-colony variants evade host clearance, are hyper-inflammatory, and persist in multiple host environments. *PLoS Pathog* 2018;14(2):e1006842. doi: 10.1371/journal.ppat.1006842
[87] Mishra M, Byrd MS, Sergeant S, et al. *Pseudomonas aeruginosa* Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response by limiting complement-mediated opsonization. *Cell Microbiol* 2012;14(1):95–106. doi: 10.1111/j.1462-5822.2011.01704.x
[88] Jensen PØ, Bjarnsholt T, Phipps R, et al. Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by *Pseudomonas aeruginosa*. *Microbiology* 2007;153(Pt 5):1329–1338. doi: 10.1099/mic.0.2006/003863-0
[89] Ciofu O, Tolker-Nielsen T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents—how *P aeruginosa* can

escape antibiotics. *Front Microbiol* 2019;10:913. doi: 10.3389/fmicb.2019.00913
[90] Moser C, Jensen PØ, Thomsen K, et al. Immune responses to *Pseudomonas aeruginosa* biofilm infections. *Front Immunol* 2021;12:625597. doi: 10.3389/fimmu.2021.625597
[91] Dobay O, Laub K, Stercz B, et al. Bicarbonate inhibits bacterial growth and biofilm formation of prevalent cystic fibrosis pathogens. *Front Microbiol* 2021;12:625597. doi: 10.3389/fmicb.2018.02245
[92] Farha MA, French S, Stokes JM, Brown ED. Bicarbonate alters bacterial susceptibility to antibiotics by targeting the proton motive force. *ACS Infect Dis* 2018;4(3):382–390. doi: 10.1021/acsinfecdis.7b00194
[93] Fiore M, Picco C, Moran O. Correctors modify the bicarbonate permeability of F508del-CFTR. *Sci Rep* 2020;10(1):8440. doi: 10.1038/s41598-020-65287-4
[94] Akong K, Chow O, Hazen S, Nizet V. Neutrophil extracellular traps require hypochlorite production via myeloperoxidase. *Am J Respir Crit Med* 2012;185:A1368. doi: 10.1164/ajrccm-conference.2012.185.1_MeetingAbstracts.A1368
[95] von Köckritz-Blickwede M, Nizet V. Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. *J Mol Med* 2009;87(8):775–783. doi: 10.1007/s00109-009-0481-0
[96] von Köckritz-Blickwede M, Chow O, Ghochani M, Nizet V. Visualization and functional evaluation of phagocyte extracellular traps. *Methods Microbiol* 2010;37:139–160. doi: 10.1016/S0580-9517(10)37007-3
[97] Neumann A, Berends ET, Nerlich A, et al. The antimicrobial peptide LL-37 facilitates the formation of neutrophil extracellular traps. *Biochem J* 2014;464(1):3–11. doi: 10.1042/BJ20140778
[98] Dorschner RA, Lopez-Garcia B, Peschel A, et al. The mammalian ionic environment dictates microbial susceptibility to antimicrobial defense peptides. *FASEB J* 2006;20(1):35–42. doi: 10.1096/fj.05-4406com
[99] Abou Alaiwa MH, Reznikov LR, Gansemer ND, et al. pH modulates the activity and synergism of the airway surface liquid antimicrobials β-defensin-3 and LL-37. *Proc Natl Acad Sci U S A* 2014;111(52):18703–18708. doi: 10.1073/pnas.1422091112
[100] Papayannopoulos V, Staab D, Zychlinsky A. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. *PLoS One* 2011;6(12):e28526. doi: 10.1371/journal.pone.0028526
[101] Marcos V, Zhou Z, Yildirim AO, et al. CXCR2 mediates NADPH oxidase-independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation. *Nat Med* 2010;16(9):1018–1023. doi: 10.1038/nm.2209
[102] Dubois AV, Gauthier A, Bréa D, et al. Influence of DNA on the activities and inhibition of neutrophil serine proteases in cystic fibrosis sputum. *Am J Respir Cell Mol Biol* 2012;47(1):80–86. doi: 10.1165/rcmb.2011-0380OC
[103] Southern KW, Patel S, Sinha IP, Nevitt SJ. Correctors (specific therapies for class II CFTR mutations) for cystic fibrosis. *Cochrane Database Syst Rev* 2018;8(8):CD010966. doi: 10.1002/14651858.CD010966.pub2

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