Communication

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Is amiloride a promising cardiovascular medication to persist in the COVID-19 crisis?

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SUMMARY In the ongoing coronavirus diseases-2019 (COVID-19) crisis that caused immense suffering and deaths, the choice of therapy for the prevention and life-saving conditions must be based on sound scientific evidence. Uncertainty and apprehension are exacerbated in people using angiotensin-converting enzyme (ACE) inhibitors to control their comorbidities such as hypertension and diabetes. These drugs are reported to result in unfavorable outcome as they tend to increase the levels of ACE2 which mediates the entry of SARS-CoV-2. Amiloride, a prototypic inhibitor of epithelial sodium channels (ENaC) can be an ideal candidate for COVID-19 patients, given its ACE reducing and cytosolic pH increasing effects. Moreover, its potassium-sparing and anti-epileptic activities make it a promising alternative or a combinatorial agent.

Keywords ACE2, ACE inhibitors, amiloride, COVID-19, cytosolic pH, ENaC

The world is anxiously watching the escalating spread of a novel pandemic that has already caused immense suffering and deaths (*1*). In this situation, the choice of therapy for coronavirus diseases-2019 (COVID-19) prevention and life-saving purpose must be supported by compelling scientific evidence (*2*). People suffering from co-morbid conditions such as diabetes and hypertension are reportedly at a higher risk of death (*3*). Uncertainty and apprehensions are exacerbated in these people since they rely on certain drugs to control their comorbidities (*4*). If at all angiotensin-converting enzyme (ACE) inhibitors result in unfavorable outcomes (*5,6*), a potassium-sparing diuretic can come to rescue. We hypothesized that amiloride, a prototypic inhibitor of epithelial sodium channels (ENaC) (*7*), can alleviate the expression of ACE2 in human lung epithelial cells.

In our study, amiloride, an epithelial sodium channel (ENaC) inhibitor, was found to reduce the expression of angiotensin-converting enzyme (ACE2) in both human alveolar epithelial and bronchial epithelial cell lines at 24 h as illustrated in Figure 1. Similarly, 'with no lysine kinase' (WNK) inhibitor showed a reduction in both cell lines but it was not significant. WNKs are a novel family of serine-threonine kinase known to regulate ENaC (*8*) and therefore it was inhibited in the study to determine the signaling pathway involved.

Multiple reasons support the use of amiloride in patients with cardiovascular morbidities during the coronavirus diseases-2019 (COVID-19) crisis. Firstly, ACE2 mediates the entry of SARS-CoV-2 (*9*) and therefore it may constitute a pharmacological target to limit cell entry of the virus (*10*). Amiloride induced reduction in ACE2 expression in bronchial and alveolar epithelial cells indicates a beneficial effect of the drug in restricting the entry of SARS-CoV-2. Secondly, a decreased cytosolic pH is believed to be the most important reason behind COVID-19 infection resulting in a higher incidence of the disease in the elderly and smokers. Amiloride can counteract the low cytosolic pH by acting on Na^+/H^+ exchanger (*11*). Thirdly, amiloride is effective in lung tissue (*11*), which is the most frequent site of infection for SARS-CoV-2 indicated by acute respiratory distress syndrome and mortality (*12*). Fourthly, potassium was reported to be significantly lower in severe COVID-19 patients (*13*) and the correction of hypokalemia was found to be challenging because of its continuous renal potassium loss resulting from the degradation of ACE2 (*14*). Amiloride with its potassium-sparing diuretic activity (*15*), helps restore normal serum potassium concentrations in those who develop hypokalemia (*16*). Finally, The Center for Disease Control and Prevention (CDC) suggests that epilepsy, among neurological comorbidities, maybe a predisposing factor for COVID-19 despite lack of evidence (*17*). Fortuitously, the illness associated with abnormal electric discharge (*18*) was found to be alleviated in rodents by anti-seizure and other neurological activities of amiloride (*19*).

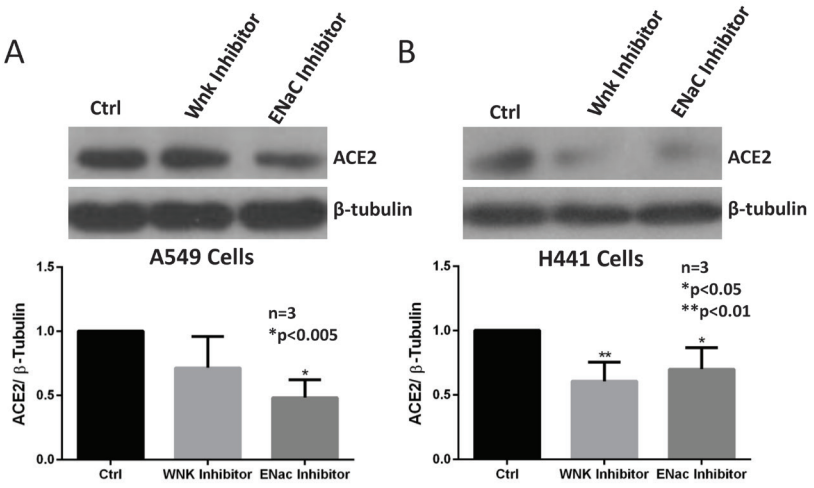

Figure 1. Amiloride suppresses ACE2 expression in A549 and H441 cells. Immunoblots and densitometric analyses of ACE2 expression on WNK and ENaC inhibitors at 24 h in **(A)** A549 and **(B)** H441 cell lines.

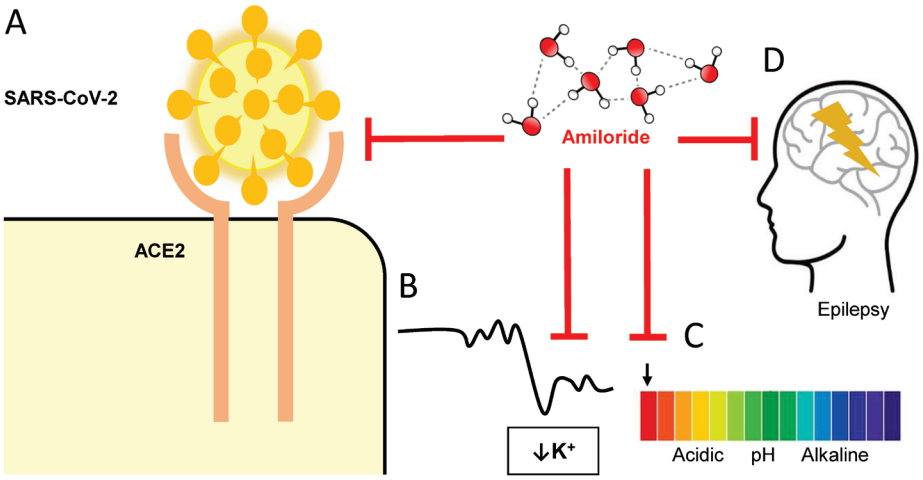

Figure 2. Diagrammatic illustration of amiloride effects. It inhibits **(A)** ACE2 expression; **(B)** Hypokalemia; **(C)** Acidic pH in the cytosol and **(D)** Seizures.

Regardless of the salt-sensitive status, large meta-analyses have reported the superiority of low-dose diuretics against other alternatives in hypertension (*20*). In the ongoing uncertainty over the safety of ACE inhibitors during the COVID-19 crisis, amiloride usage as an alternative or a combinatorial agent in the aforesaid co-morbidities can not only ameliorate the cardiovascular events but can also help in COVID-19 as pictorially illustrated in Figure 2.

Materials and Methods

Cell lines and reagents: The human A549 adenocarcinoma (ATCC® CCL-185™) and Human bronchial (H441) epithelial (ATCC® HTB-174™) cell lines obtained from ATCC (Manassas, VA) were cultured in DMEM high glucose and RPMI 1640 media (Hyclone, Logan, UT), respectively supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals, Atlanta, GA), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified incubator at 37°C and 5% CO_2. Cells were routinely passaged when they reached 80-90% confluency. Compound inhibitors were obtained from Selleckchem, Houston, TX for WNK (WNK 463, Cat#S8358) and epithelial sodium channel (Amiloride, Cat#S1811) to be used in the concentrations of 1 µM and 10 µM, respectively.

Western blotting: The cell lysates were prepared using 1X RIPA lysis buffer (Millipore, Temecula, CA) supplemented with protease and phosphatase inhibitor tablets (Roche Applied Science, Indianapolis, IN). Protein concentration was measured by the DC protein assay (Bio-Rad Laboratories, Hercules, CA) and approximately 40-50 µg of cell lysates in Laemmli buffer were used. Densitometry was performed using NIH ImageJ software. Primary antibodies against ACE2 (Cat# MABN59) and β-tubulin (Cat#2118S)

were purchased from Millipore Sigma (Burlington, MA) and Cell Signaling Technology (Danvers, MA), respectively.

Statistical analysis: All the data are presented as mean ± SEM. The 'n' value for each figure implies the number of samples in each group. All the data were analyzed by parametric testing using the Student's unpaired *t*-test or one-way ANOVA, followed by the posthoc test using the GraphPad Prism 6.01 software. Data with $p < 0.05$ were considered significant.

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Conflict of Interest: Authors declare that there are no financial or other conflicts of interest exist.

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