https://doi.org/10.31925/farmacia.2020.5.1 **REVIEW**

LYSOSOMOTROPIC PROPERTIES OF SODIUM BICARBONATE AND COVID-19

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Abstract

SARS-CoV-2 causing COVID-19 has appeared as an ongoing global public crisis, growing with geometric progression and has caused huge devastation till date majorly because of lack of targeted therapeutic agents like vaccines. SARS-Cov-2 entrance into the host cells is reliant on acidic pH. Thus, in the current clinical emergency there is a pressing need to look forward for adjunct therapies which could counter the acidic pH, so as to restrain the viral entry and its subsequent reproduction in the host cells. Therefore, the current review attempted to explore the possibility to use sodium bicarbonate as an alternative lysosomotropic agent based on the reported literature owing to its anti-flu properties and widespread use during 1918 Spanish flu pandemic. The suggestions put forward in the current review article based on the careful use of sodium bicarbonate could probably help to restrain SARS-CoV-2 infection.

Rezumat

COVID-19 a generat o criză de sănătate publică globală cu consecințe uriașe până în prezent, în principal din cauza lipsei agenților terapeutici specifici. Pătrunderea SARS-Cov-2 în celulele gazdă depinde de pH-ul acid. Astfel, în situația clinică actuală este nevoie de terapii adjuvante care ar putea contracara pH-ul acid, pentru a limita penetrarea virusului la nivel celular. Prin urmare, studiul de față explorează posibilitatea utilizării bicarbonatului de sodiu ca agent lizozomotrop alternativ, pe baza datelor referitoare la gripa spaniolă din 1918. Sugestiile prezentate în actualul articol, privind utilizarea bicarbonatului de sodiu, ar putea ajuta contribui la limitarea infecțiilor cu SARS-CoV-2.

Keywords: coronavirus, sodium bicarbonate, treatment, SARS-CoV-2, COVID-19

Introduction

Coronavirus Disease 2019 (COVID-19) has emerged as the most recent dreadful public health crisis that is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Initially it originated from Wuhan, Hubei province, China during December 2019 and has now gripped almost all the countries of the world (> 210) countries and the number is still increasing [1, 2]. Due to lack of any specific drugs for treatment or prevention of SARS-CoV-2, we can see an alarming ongoing surge in the number of positive cases turning up across the globe. As of 8th August, 2020, there are approaching 20 million laboratory confirmed cases and more than 727,009 deaths caused by SARS-CoV-2 infection [3]. Due to lack of targeted therapeutic agents against this virus, the number of people affected by this nightmare pandemic infection are growing with geometric progression and if the situation is not contained immediately, the number can grow further higher and higher, probably 1 million positive cases *per* week which can eventuality lead more deaths [4, 5]. The viral infection of SARS-CoV-2 spreads at a faster rate but with lower fatality rate between 2 - 4% than its previous cousins known as SARS-CoV and Middle Eastern respiratory syndrome (MERS-CoV). The major transmission route is *via* air through micro droplets (Droplet infection) and closer contacts between animals (Bats)-to-human initially and now from human-to-human or can happen even *vice-versa* (human-to-animals) [6, 7]. The basic physiological indications of COVID-19 affected individuals initially include symptoms such as cough, fever, sore throat, fatigue, breathlessness etc.
Nevertheless, in severe cases it leads metabolic acidosis, pneumonia, acute respiratory distress syndrome (ARDS), multi organ dysfunction, bleeding disorders and septic shock [1, 2, 8]. COVID-19 pandemic related deaths occur due to hyper inflammation of respiratory system

in the form of cytokine storm and this is considered as a major reason for surge in the death cases [9].

SARS-CoV-2 virus commonly known as Coronavirus-2 is a positive ssRNA virus (*Beta-coronaviruses*) which has a large sized genome containing 30,000 bases and 15 genes, probably a chimeric virus. ssRNA of SARS-CoV-2 is susceptible towards mutation and surprisingly its mutation rate seems to be < 25 mutations *per* year as compared to seasonal flu with mutation rate of 50 mutations/year [6]. The chances for the development of new SARS-CoV-2 strains with variability in their virulence seems minimal as compared to seasonal flu, this is because SARS-CoV-2 does not mutate so rapidly as they possess polymerase (replicase) based 3` exonuclease proof reading activity [1, 5, 10]. Therefore, development of future vaccine seems to be a promising strategy to circumvent it efficiently. However, currently people are suffering a lot because of lack of any specific therapeutic agents to control this pandemic, and if the alternative therapies are not recommended then the situation can change from bad to the worst. It is a good sign that SARS-CoV-2 does not show much variation in the receptor-binding domain of entry protein known as Spike or S protein which could be the major target for future therapeutics including vaccines [5, 9].

Keeping above grim figures into consideration and our curiosity to share a strategy which could be within the reach of every individual, the authors attempted to convey through this review article before scientific community and medical professionals about probable capacity of baking soda to restrain SARS-CoV-2 viral entry and its downstream pathogenesis. This is because any kind of prophylactic or therapeutic interventions could save the lives of millions of severely ill helpless and needy patients.

Molecular targets of SARS-CoV-2

A proper understanding of how SARS-COV-2 enters and later infects the cells will have major implications towards development of effective treatment for prevention/treatment of SARS-CoV-2. The studies till date have demonstrated that with the help of spike (S) protein present on the surface of SARS-CoV-2 (ligand), it recognizes and binds the target cell receptor (e.g. enterocytes, pneumocytes, kidney epithelial cells, immune cells, etc.) known as angiotensin converting enzyme-2/ACE2 [7]. Many experimental findings have revealed that ACE2 specific antibodies of SARS-CoV might partially block entry of SARS-CoV-2 (Figure 1), indicating role of ACE2 receptor in viral entry [11-13]. The most of the amino acid residues (both in SARS-S and SARS-2-S) responsible for binding with ACE2 receptor of target cells are conserved [11]. Entry of SARS-CoV-2 into the cells takes place with the help of serine protease known as transmembrane protease, serine 2/TMPRSS2 for S protein priming, thus TMPRSS2 seems to be a promising target (Figure 1) for therapeutic agents (e.g. chamostat mesylate) in order to prevent spread of virus and its subsequent pathogenesis using lopinavir/ritonavir and remdesivir [11-13].

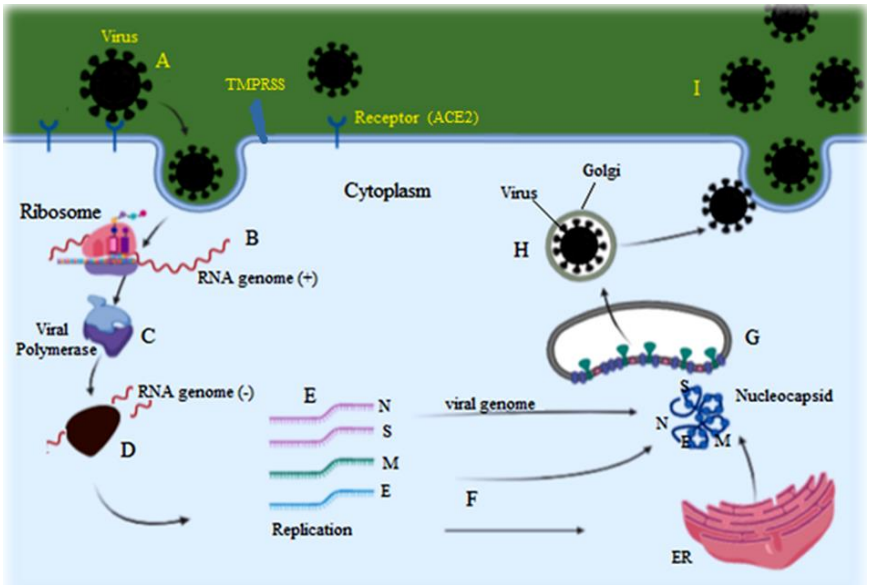

Figure 1.

Entry and replication cycle of Novel Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2)
(A) Binding of virus with receptor, membrane fusion and endocytosis; (B) Viral genome released; (C) Viral polymerase protein translation; (D) RNA Replication; (E) Sub-genomic Transcription; (F) Viral structural protein translation; (G) Spike protein, Membrane protein and Envelope protein combine with nucleocapsid; (H) Mature virion formation; (I) Exocytosis. Where: N: Nucleoprotein; S: Spike protein; M: Membrane protein; E: Envelope protein.

pH dependent viral entry of SARS-CoV-2

Like other coronaviruses (SARS-CoV), entry of SARS-CoV-2 into a host cell seems to be pH dependent; because once a virus fuses with a human cell *via* S-glycoprotein then its entry inside the cell utilizes a pH-dependent endocytic pathway [14]. When these endo-lysosome vesicles move towards the nucleus, their pH drops (more acidic), which catalyses fusion of viral and cell membranes [15]. The studies have mentioned that there is more reduction in viral entry if alkaline conditions are retained in the host cells i.e. pH > 7, while as under acidified conditions (pH < 7) there is more viral load inside the host cells [7, 16, 17]. Therefore, it is evident that novel therapeutic strategies could be designed to increase the pH (alkaline) of endo-lysosomes through infusion of pH increasing lowering agents known as lysosomotropic agents. They are defined as weaker bases that have potential to penetrate lysosomes in their protonated form and thus increase their intracellular pH [18]. The use of safer lysosomotropic agents could pave a way to act as one of the effective counter strategy to thwart infection caused by SARS-CoV-2.

Keeping the concept of lysosomotropic agents in mind, few researchers have recently advised to use anti-malarial drug chloroquine (hydroxychloroquine) as potential option to cure COVID-19 because it accumulates in the acidic endo-lysosomes and thus increasing their pH which is required to block the entry and further pathogenesis of SARS-CoV-2. The increase of pH of endosomes probably inhibits the protease activities of TMPRSS2 and thus prevents the cleavage of S protein [11, 13, 15, 17, 19]. Although the clinical trial conducted by Gautret *et al.* [19] had some of research design issues with lesser number of subjects enrolled, nevertheless, it coincides with the basic idea how a lysosomotropic agent could be used to restrain viral entry and subsequent reproduction cycle through neutralization of acidic endo-lysosomes. The idea to use chloroquine (hydroxychloroquine) against SARS-CoV-2 as an off-label treatment is still controversial between the researchers, as no conclusive study has recommended it as a safer and effective agent. The use of long term and higher doses are reported to cause severe retinopathies and other unusual side effects [13, 20, 21]. Moreover, use of bafilomycin and ammonium chloride (NH_4Cl) in *in vitro* cells (Vero E6) has demonstrated that ACE2 receptor was affected by elevating endosomal pH which prevented viral entry into the host cells [17]. Thus, the drugs prescribed against enveloped viruses increase intra-cellular pH (alkalinity within the cell) there by reducing the activity of pH-dependent viruses. However, they can provoke negative side effects like nausea, retino-pathy, cardiomyopathy, neuromyopathy, psoriasis and porphyria [22]. Sodium bicarbonate (SB) has been considered as a safe and effective therapeutic agent when given in the recommended doses and is relatively free of serious side effects [23]. It is an ideal choice of buffer solution in different metabolic and respiratory acidosis such as lactic acidosis, ketoacidosis, drug toxicities and acidosis due to pathogens [23, 24].

Mechanism of action of sodium bicarbonate

Human body has an in-build homeostatic bicarbonate buffer system (HCO_3^-) which regulates pH in blood and cells that control metabolic functions. During acidic environment it acts as the proton acceptor and converts to carbonic acid (H_2CO_3) which by reversible process forms CO_2 and H_2O. When the environment turns more alkaline it releases proton forming back to bicarbonate (HCO_3^-). This reversible process is facilitated by the enzyme called carbonic anhydrase (CA) present in blood, stomach, pancreas and kidneys. CA belongs to the family of metalloenzymes consisting of five classes (α, β, γ, δ, ζ) of which α-class is primarily found in mammals [25]. Apart of its role in pH regulation, it also plays its part in other metabolic processes such as gluconeogenesis, lipogenesis and ureagenesis [26]. Two major by-products of this acid-base buffering system are CO_2 and (HCO_3^-).

Approximately 70% of CO_2 is converted to bicarbonate in the blood while the remaining is exhaled by the lungs. The (HCO_3^-) combines with free H^+ ions maintaining the pH balance in the blood. An experimental set-up in rabbit ventricular monocytes showed the role of this CO_2/(HCO_3^-) buffer system in regulation of H^+ mobility that maintain uniform pH [27]. The biochemistry of this reversible process follows Le Chatelier principle which states that if more H^+ is present the process shifts to backward so that more reactants are formed to maintain equilibrium.

$$H_2O + CO_2 \xrightleftharpoons{CA} H_2CO_3 \xrightleftharpoons{CA} H^+ + HCO^{3-}$$

Administration of sodium bicarbonate is needed when there is metabolic/respiratory acidosis in the patient. Metabolic acidosis is a condition when blood pH < 7.35 due to reduced bicarbonate (HCO^{3-}) levels in the blood those further worsen with the decrease in arterial partial pressure of carbon dioxide ($PaCO_2$). For every 1 mmol/L fall in serum HCO^{3-} there will be ~ 1 mmHg decrease in $PaCO_2$ that will fall the blood pH < 7.20 [28]. According to Henderson-Hasselbalch method, metabolic acidosis is a state when concentration of (HCO_3^-) is below 20 mmol/L in blood plasma [29]. Respiratory acidosis is a state of hypoventilation that may be due to pulmonary diseases, drug overdose, obesity and brain injury. Severe respiratory acidosis leads to acute respiratory distress syndrome (ARDS) depicting the lungs insufficiency to exhale CO_2 and exchange of O_2 across alveolar membrane [30]. Thus human body enters into the state of acidosis when there is loss of (HCO_3^-) due to renal dysfunction or the presence of

acids in the gastrointestinal tract that neutralize the (HCO_3^-) ions or other pulmonary diseases. The imbalance in the acid-base results in the adverse effects that include arterial dilation with hypotension, decrease in cardiac output, affecting the metabolic process e.g. ATP production and the body's immune system [31].

Table I

Bicarbonate buffer system values under acidosis [32, 33]

Parameters	Normal Condition	Metabolic Acidosis	Respiratory Acidosis
HCO_3^-	4 - 22 mM	< 22 mM	< 22 mM
$PaCO_2$	35 - 45 mmHg (6.7 ± 0.34 mM)	< 35 mmHg	> 45 mmHg
pH	6.2 - 7.22	< 6.2	< 6.2

Bicarbonate (HCO_3^-) ions are impermeable to membrane and their movement is facilitated by specific integral membrane proteins. There are 3 groups of bicarbonate transport protein: SLC4A $Cl^-/(HCO_3^-)$ exchanger, SLC4A sodium coupled transporter, SLC26A (Figure 2). Bicarbonate ions when combine with transport protein form metabolons and CA increase their activity of transport [34]. The bicarbonate ions (HCO_3^-) in the sodium bicarbonate has the tendency to displace the Cl^- ions in extracellular fluid (ECF) that will increase the strong ion difference (SID) in ECF leading to alkalization. This Cl^-/HCO_3^- ions exchange is done through the transport family protein called solute carrier family (SLC4A1/A2/A3). SLC4A1 exchanges the Cl^- with HCO_3^- across plasma-membrane for re-absorption of HCO_3^- into the blood [35]. Mutations in the protein part of SLC4A1 lead to distal renal tubule acidosis, lowers the pH of urine which ultimately leads to metabolic acidosis [36]. SLC4 sodium independent transport proteins facilitate 1:1 transport of HCO_3^-/Cl^- across membrane and thus maintaining the anion gap ($Na^+/Cl^-/HCO_3^-$) across the membrane [37]. Other protein transporters belonging to the family of SLC4 include sodium driven Cl^-/HCO_3^- exchanger (NBCs). NBCs are grouped into two categories: electro-neutral (NDCBE/NBCn1/NBCn2) and electrogenic (NBCe1/NBCe2) which are expressed on brain, kidney and liver [38, 39]. NBCe1 has been seen to play role in the renal acid-base balance and the mutation in this co-transport system leads to the proximal renal tubule acidosis [40]. The NDCBE/NBCn2 is the predominant co-transporters Na^+/HCO_3^- in the brain regulating intra-cellular pH and the pH homeostasis across the blood brain barrier (BBB). These two transporters in the brain drive the movement of $1Na^+/2HCO_3^-$ inward while $1Na^+/3HCO_3^-$ outward from cerebrospinal fluid (CSF) to blood [41]. NBCe2 co-transporter present on the membrane of brain, liver and kidney mediates Na^+/HCO_3^- influx in the ratio of 1:2 or 1:3 thus maintaining intracellular pH balance [42]. In the small intestine NBCe1 and CFTR co-transporters are involved in the HCO_3^- and mucus secretions in response to the acidic environment. Study of the intestinal tissue in mice model showed the incapability of mucus secretion in CF (cystic fibrosis) disease when the HCO_3^- secretion is compromised while the treatment of $NaHCO_3$ saline solution increased the CFTR and NBCe1 functioning in the tissue model [43]. Thus treating acidosis with administration of sodium bicarbonate affects various cellular and metabolic processes in different tissues.

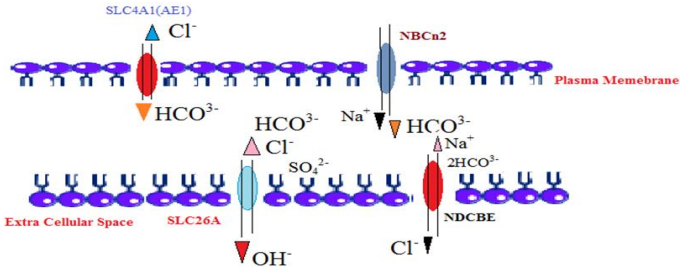

Figure 2.

The mechanism of action of bicarbonate transport proteins for the influx/efflux of the HCO_3^-, Na^+ and Cl^- ions through the plasma membrane.

Probable effects of sodium bicarbonate to circumvent COVID-19 viral infection

Respiratory infections are a significant reason for flu like sickness manifestations among affected human populations, prompting considerable dreadfulness and mortality every year [44]. At present the flu vaccinations used are only convincing where the virus strains anti-genically match the virus strains. Thusly, regular flu vaccines must be refreshed every year. Regular flu vaccines neglect to manage the concrete protection against antigenically particular pandemic flu viruses [45]. The successive changes, in the viral antigenic structure, present troubles in the advancement of antibody particularly for RNA viruses. Besides, during rise of a recently dreadful coronavirus, creation of vaccine requires time and may be ineffective as well. This gap between new dreadful COVID-19 viral strain appearance and vaccine production has already caused catastrophic

death of more than seven hundred thousand people and millions may lose their lives, if it is not contained now. Since there is no completely compelling medicine or immunization for this viral disease, looking for alternative treatments is a sensible approach.

Coronavirus cell fusion occurs at acidic pH and was found to be stable at pH = 6 at 37°C (half-life = 24 h) [46, 47] and it was found that the attack of the virus was irreversibly inactivated by treatment at pH = 8 at 37ºC (half-life = 30 min) and this coronavirus pH-dependent thermolability is because of the result of conformational changes in the corona virus peplomer [47, 48]. Diet plays an important role in adjustment of body pH. Sodium bicarbonate drinks, diets etc. can cause a very small change in blood pH within a range considered as normal. In slightly higher pH (alkaline) where the body environment becomes alkaline, the viruses might be weakened. Thus, reducing acidic diets and increasing the intake of alkaline food sources can help in alkalization [49]. An alkaline body can absorb up to 20 times more oxygen than an acidic body and it enhances immunity of body to kill microbes. The huge surge in the laboratory confirmed that COVID-19 cases are higher in the western countries; probably the typical Western diet is an acid-forming diet, low in the valuable alkaline minerals [50]. Large number of flu cases were studied and observed in laboratory and it was analysed that there was a decrease in bicarbonate level and other bases in the blood plasmas as well as in tissues. Further, it was concluded that flu is a local manifestation of a systemic disturbance, like change in alkalinity or it could be called a mild acidosis, which mostly occurs due to a decrease in bicarbonate content in blood. The symptoms of flu swiftly decreased upon the administration of sodium bicarbonate in large doses by mouth and by rectum [51]. In 1918 and 1919, while battling "influenza", it was brought to the consideration of the US Public Health Service that seldom any individual who had been altogether alkalinized with sodium bicarbonate has got the illness, and the individuals who contracted it, whenever alkalinized early, would constantly have mild attacks [49]. Sodium bicarbonate (henceforth called as SB) is used in patients with renal tubular acidosis syndromes, diarrhoea, acute lactic acidosis and ketoacidosis and is commonly used as a pH buffering agent [52]. It has been found that the changing acidic pH in pulmonary tuberculosis (TB) lesion may affect growth of TB bacilli. Previous studies have found that the effect of adjuvant inhalation of sodium bicarbonate (SB) 8.4% in smear-positive pulmonary TB to standard anti-TB drugs accelerates smear conversion, culture conversion, and clinical and radiological improvement [53, 54]. Patients with renal tubular acidosis syndromes or diarrhoea are given chronic bicarbonate replacement therapy. In patients with acute lactic acidosis and keto-acidosis, however, bicarbonate therapy is always individualized [52].

Rhinoviruses and coronaviruses are classified as pH dependent viruses as they attack and infiltrate host cells by fusion with cellular membranes at low pH. There is fusion dependency between the viral and cellular membranes [55, 56]. The barrier to this is plasma membrane which does not allow the viruses and parasites to invade. To enter the host cell and use its machinery, the virus has to cross this barrier. For enveloped viruses there must be fusion between membrane and virus and it occurs *via* endocytic pathway, the fusion depends on endosomal compartments. These cellular compartments at low pH are responsible for triggering conformational changes in the glycoproteins of the virus [55, 57]. As pH drops there will be a rapid fusion e.g. Ebola virions which at low pH move to more acidic compartment and later on late endosomes help in pH dependent fusion. Earlier these virions move by pinocytosis by forming macropinosomes and fuse with other vesicles of the standard endolysosomal pathway [58]. Pharmaceutical and biotechnology companies around the world are working tirelessly for development of anti COVID-19 vaccines. However, experts predict it will take minimum one year to be available in the market [6]. The metabolic acidosis can be managed using sodium bicarbonate intravenously between 7.5 - 8.4%, while as over-dosage can lead other metabolic abnormalities [23, 59-62]. It was found through a study that 7.5 - 8.4% sodium bicarbonate is safe for human body with no considerable side effects [23, 54, 63], an intravenous infusion of SB lead significant inhibition of lower respiratory tract pathogens like bacteria, fungi and mycobacteria [54]. A cohort study regarding the oral rinse of sodium bicarbonate showed significant increase in salivary pH and prevented overgrowth of acid uric bacteria [64], so further validates the role of baking soda to restrain reproduction of these microorganisms.

HCO_3^- ions secretions in respiratory tract not only maintain the pH but also protect the epithelium from different inhaled pathogens. Aerosol inhalation of $NaHCO_3$ (100 mmol/L) in a bacterial culture experiment showed promising results in inhibiting bacterial growth and cAMP reduction of prevalent cystic fibrosis pathogens [65, 66]. $NaHCO_3$ combinations with different drugs favour their uptake in gastrointestinal tract by making the medium more alkaline. In an experiment studying the effect of drug combinations on fungal pathogens, $NaHCO_3$ (40 mM) + quinine (2 mM) or hygromycin (7.5 µg/mL) for 24 h showed synergistic effects by decreasing the fungal metabolic activity with more than 60% [67]. The normal pH of extracellular spaces supports body immune system for fighting pathogens. More acidic environment affects the immune response by modulating the various signalling molecules such as H_2O_2, complement system and neutrophils [68-70]. *C. elegans* as model organism for studying human immune system showed that adding $NaHCO_3$ (25 mM, pH = 7) as buffering solution reduced

the activity of pathogen in acidic intestine [71]. Thus, sodium bicarbonate ($NaHCO_3$) is an ideal choice of buffer system to maintain extracellular pH as it mimics the role of body's own bicarbonate HCO_3^-. But the overdose of it can lead to Na^+ overload and metabolic alkalosis that may cause fluid retention, hypertension, heart failure and renal failure [34].

Coronavirus contamination is highly sensitive to pH as it has been found that MHV-A59 strain of coronavirus is quite stable at pH 6.0 (acidic). Other human coronavirus strain 229E is extremely infective at pH 6.0 and is rapidly and irreversibly inactivated by brief treatment at pH 8.0 (alkaline). Murine coronavirus A59 at pH 6 (acidic) produces ten times more infection than virus. The acidic pH 5 - 6 is maintained by endosomal membrane (proton pumps) which is responsible for triggering reaction between virus and endosomal membrane [72, 73]. In order to release the individual viral ribonucleoproteins (vRNPs), low pH exposure is also necessary [74]. Severe metabolic acidosis and intracellular acidosis is reported one week after disease onset by 2019 novel coronavirus [8, 75-77]. In a cohort study on COVID-19 in China, it has been found that 30% non-survivors had acidosis as compared to survivors with only 1% reported acidosis [78]. The correction of such acidosis could not be ignored in patients with COVID-19 [79]. In conclusion, in the current clinical emergency sodium bicarbonate seems to be the preferable way to increase pH as it has been known as far back as the Spanish Flu pandemic of 1918 to save lives [80].

Conclusions

Novel coronavirus entry into host cells is pH dependent and has caused global crisis, majorly due to lack of targeted therapeutic agents. The probable off-label treatment of chloroquine or hydroxychloroquine as lysosomotropic agent to control viral replication is still controversial, as its higher recommended doses are reported to cause unusual side effects like retinopathy. Similarly, role of other antiviral agents is yet to be established. Sodium bicarbonate, a lysosomotropic agent has been reported to be involved in neutralization of acidosis, thus it could be largely seen as a safer and easily available option for possible prophylactic and therapeutic interventions against SARS-CoV-2 replication and pathogenesis.

Conflict of interest

The authors declare no conflict of interest.

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