Brief Report

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TANAFFOS

Local Administration of Sodium Bicarbonate for Preventing COVID-19 Associated Mucormycosis

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Background: One important complication of the coronavirus disease 2019 (COVID-19) is COVID Associated Mucormycosis (CAM), especially in patients with conditions such as diabetes and in immunosuppressed patients. Systemic acidosis, hyperglycemia, and other biochemical factors such as free iron and β-hydroxybutyrate (BHB) can play a role in this complication.

Materials and Methods: *Rhizopus oryzae* was isolated from a patient at Masih Daneshvari Hospital microbiology laboratory and sub-cultured on the Potato Dextrose Agar (PDA) for 48 hours at 37 °C. Subsequently, Roswell Park Memorial Institute (RPMI) 1640 Broth medium buffered to pH 7.0 with 3-N-morpholino-propane sulfonic acid. Macrodilution and microdilution methods were performed with 8.4% sodium bicarbonate. After 24 hours of incubation at 35°C, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentrations (MFC) were evaluated.

Results: We found that the minimum inhibitory and fungicidal concentrations are at 1.05 % and 2.1 % respectively. Therefore, the minimum concentration is 2% sodium bicarbonate, which requires achieving the desired environmental pH for fungal inhibition and fungicidal effects.

Conclusion: Regulation of systemic acidosis by sodium bicarbonate could be used to decrease the chance of mucormycosis. In addition, According to our study and some others, an alkaline environment can prevent fungal growth. We found that a minimum concentration of 2% sodium bicarbonate is required to achieve the desired mucosal pH to inhibit the fungus. Therefore, sodium bicarbonate inhalation, as a cost-effective and well-tolerated medicine, is a good candidate for the prevention of mucormycosis. In this regard, extensive clinical and laboratory research is needed to achieve more accurate doses and appropriate administration intervals.

Keywords: Sodium Bicarbonate; COVID-19; Mucormycosis

INTRODUCTION

Since the onset of the COVID-19 pandemic, various complications of the disease have been reported to play a role in the prognosis of morbidity and mortality. One of these cases is the occurrence of CAM, especially in the delta variant, predominantly in males, diabetics and individuals who receive immunosuppressive drugs such as corticosteroids and tocilizumab (1).

Mucorales, the fungus causing mucormycosis, binds to glucose-related protein 78 (GRP78), which is a receptor located at the surface of endothelial cells of the host by expressing a spore Coat Homolog (CotH) protein. . The main feature of mucormycosis is the ability of the organism to rapid and aggressive vascular invasion, which leads to hematogenic dissemination and subsequently vascular thrombosis and parenchymal necrosis (2). An

acidic environment, BHB, hyperglycemia and iron play an important role in the virulence of mucormycosis (2, 3). Free iron plays a major role in reducing immune system reactions and low pH by impairing the ability of transferrin to chelate iron, amplifies fungal growth. In addition, BHB which is the main product of diabetic ketoacidosis (DKA) indirectly inhibits iron chelation by transferrin and can also destroy the ability of neutrophils to defend against Rhizomes (4). It seems that sodium bicarbonate can protect vascular endothelium against rhizopus invasion, by inducing alkaline pH and increasing iron chelation, as well as inhibiting the release of iron from the transferrin (2, 3). Moreover, laboratory studies confirm that bicarbonate compounds can play an inhibitory role on fungal growth and development. Review of sodium bicarbonate's effect on skin superficial fungi and potassium bicarbonate on plant pathogenic fungi; illustrate this point (5, 6). Therefore, timely administration of bicarbonate with dual effect on both in-vivo biochemical reactions and fungal growth environment can alter host-pathogen interactions and attenuates mucormycosis (3).

On the other hand, current antifungal therapies are expensive, may be toxic, and most patients require extensive surgical debridement. Therefore, easy and safe preventive strategies can be a lifesaver for patients at risk of mucormycosis (7). In order to achieve methods of preventing mucormycosis, we investigated the antifungal activity of sodium bicarbonate against mucormycosis.

MATERIALS AND METHODS

In order to evaluate the in-vitro antifungal activity of sodium bicarbonate, Broth microdilution and macrodilution methods were performed according to Clinical and Laboratory Standards Institute (CLSI) M38Ed3 standard. *Rhizopus oryzae* isolated from a patient at Masih Daneshvari Hospital microbiology lab (Ibresco, Iran) was subcultured on the PDA for 48 hours at 37 ◦C before testing. Subsequently, RPMI 1640 Broth medium buffered to pH 7.0 with 3-N-morpholino-propane sulfonic acid (MOPS; Sigma). In macrodilution test, 2 cc of sodium bicarbonate 8.4 % (Caspian Tamin Pharmaceutical Co., Rasht, Iran) was diluted with RPMI 1640 Broth medium and incubated with suspensions of *R. oryzae* with optical density (OD) ranged of 0.15 to 0.17 at 35 ^{0}C for 24 h. The MIC was defined as the first concentration of sodium bicarbonate with a 100% reduction in fungal growth.

In the microdilution test, 100 µl sodium bicarbonate 8.4% (Caspian Tamin Pharmaceutical Co., Rasht, Iran) diluted by RPMI 1460 broth medium and inoculated by 100 µl suspensions of *R. oryzae* with optical density ranged from 0.15 to 0.17. The MIC endpoint was visually determined after 24 h of incubation at 35°C and defined as a 100% reduction in fungal growth (8).

To determine the MFC, 1000 µl of broth aliquots were taken from each tube with an extract concentration equal to or higher than the MIC values and incubated in Sabouraud dextrose agar (SDA) at 35 °C for 24 h. MFC was determined as the lowest concentration that showed no fungicidal growth in the subcultures. Each experiment was performed in duplicate.

RESULTS

Liquid media micro- and macro- dilution techniques were performed on fungal isolates, confirmed the antifungal activity of sodium bicarbonate and allowed us to specify the MIC for the different concentrations of fungi studied with reproducible MICs for each isolate. MIC was observed in the 3rd tube and well 3 with the achieved concentration of 1.05% of sodium bicarbonate for both liquid media micro-and macro- dilution testing.

In the solid media (PDA) experiment, sodium bicarbonate showed a dose-dependent fungicidal activity. A concentration of sodium bicarbonate 2.1% (2th plate) presented fungicidal activity against the strain that tested (Figure 1).

Figure 1. Results correspond to the dose-dependent fungicidal activity of sodium bicarbonate at prepared concentration of 2.1% on Sabouraud dextrose agar (SDA) after incubation at 35 °C for 24 h.

DISCUSSION

The use of sodium bicarbonate, both systemically and topically, has long been used as a medicine in various diseases, and numerous researches have been done on its effectiveness and safety.

Nebulization of sodium bicarbonate induced alkaline pH in the respiratory airway, but there was no significant difference of exhaled Carbon Dioxide (CO_2) was found (9). Nebulization of sodium bicarbonate and other alkaline compounds is performed with various therapeutic benefits and is usually safe, with acceptable tolerance (10). In Cystic fibrosis patients it has been shown that the inhalation of sodium bicarbonate directly and temporarily raises the pH of airway mucosa and also decreases the viscosity of the sticky sputum (11). Considering the most mucor invasion happened through the respiratory mucosa, airway alkalization seems to be a logical strategy to provide a preventive environment for opportunistic mucormycosis. Inhalation of sodium bicarbonate has been used as therapeutic adjuncts in different infectious diseases. In this regard, the safety and inhibitory effect of nebulized sodium bicarbonate 8.4% on the growth of bacteria, fungi and mycobacteria has been observed. Especially in pulmonary tuberculosis which has been shown to significantly reduce culture conversion time at 8.4% concentration (12, 13). In addition, according to some studies performed on COVID-19 patients in Iran and India, it has been shown that sodium bicarbonate has the ability to prevent the virus from entering the respiratory mucosa and blocking pH-dependent membrane fusion (14, 15).

Our findings on both liquid media micro-and macro-dilution techniques results revealed the nature of the antifungal activity of sodium bicarbonate, whether it was fungistatic or fungicidal. The evaluation of the MIC and MFC showed that the MIC was observed at a concentration of 1.05 % and MFC at a concentration of 2.1 % of sodium bicarbonates. Therefore, it seems that a concentration of more than 2% of sodium bicarbonate is required to achieve the desired mucosal pH. As the inhaled form of sodium bicarbonate solutions through a Naso-Oral delivery apparatus can cover the upper and lower respiratory mucosa, it can be used in high risk hospitalized patients with COVID-19 for preventing CAM.

Fortunately, the concentrations of sodium bicarbonate solutions that are inhalable and available in the world pharmaceutical market are 7.5% and 8.4% (16), thus, it seems that they are able to produce a suitable mucosal pH. Although inhaled sodium bicarbonate is safe (17), whenever mucosal irritation and bronchospasm occur, the use of bronchodilators before nebulization of sodium bicarbonate may prevent bronchial hyperactivity (18). In outpatient conditions and after discharge from the hospital, a suitable and practical method is to use bicarbonate nasal spray. The use of the nasal form is important because the most commonly reported cases of CAM are rhino-sinusal and rhino-orbital, respectively (1). Existing bicarbonate nasal sprays, which are often used for rhinosinusitis and dryness of the nasal mucosa, have a low concentration of sodium bicarbonate less than 2% (https://www.dailymed.org) while a concentration of more than 2% of sodium bicarbonate is required for CAM protection.

Paranasal cavities and the sino-orbital region are the most important organs involved in mucormycosis. With further studies, the regrowth of residual mucormycosis after surgery and debridement of rhino-sinusal mucormycosis may be prevented by appropriate alkalinization of this mucosa.

CONCLUSION

Hence, modification of systemic acidosis as well as local alkalization by sodium bicarbonate can reduce the virulence of the fungus and subsequent complications and mortality in patients with CAM. In order to achieve the desired concentration and proper use interval, future extensive studies need to be performed on clinical specimens and the mechanism of action of sodium bicarbonate and its interactions with antifungal agents should be investigated.

Competing interests

Not applicable.

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