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RESEARCH ARTICLE

Exploring Chemotherapeutic Agents as Countermeasures against Respiratory Viruses: Antiviral Potential of Sugar Alcohols

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Abstract

The emergence of respiratory viruses has been attracting considerable interest due to their potential to cause pandemics, such as the 1918 Spanish flu, the 2019 Coronavirus disease, and recently the Respiratory syncytial virus (RSV) in pediatric populations. There is a critical need to identify potential agents that can be included as part of the countermeasures to aid in the preparedness for a rapid public health response in case of a pandemic. This study aimed to explore the antiviral potential of sugar alcohols against respiratory viruses with pandemic potential.

Methods: The antiviral activity of three sugar alcohols commonly utilized in the food and pharmaceutical industry, namely sorbitol, erythritol, and xylitol, were evaluated against Influenza (H1N1), RSV (A2), and SARS-CoV-2 (B.1.617.2; Delta) via a highly differentiated, three-dimensional, in vitro model of normal, intact, human-derived tracheal/bronchial epithelial cells. The sugar alcohol solutions were tested at a 5% concentration in duplicate inserts of the three-dimensional tissue models of the human airway.

Results: Antiviral activity was measured in virus yield reduction assays by calculating the log reduction value defined as the average reduction of virus compared to the average virus control on day 3 (Influenza), day (RSV), and day 6 (SARS-CoV-2) after infection. Antiviral agents utilized as comparators were Ribavirin (Influenza, RSV) and Remdesivir (SARS-CoV-2). Erythritol displayed antiviral efficacy against Influenza with a log reduction value of 3.17. RSV was effectively inactivated by both sorbitol and xylitol with 2.49 and 2.65 log reduction values, respectively. All tested sugar alcohols inactivated SARS-CoV-2 Delta with a median log reduction value of 3.50.

Conclusion: The results of this study suggest that alone or in combination, sugar alcohols can inactivate respiratory viruses known to have pandemic potential. Additional research is needed to advance the development of sugar alcohols as chemotherapeutic countermeasures against other pandemic respiratory viruses.

Keywords: *Sugar Alcohols, Antiviral, Respiratory syncytial virus, Influenza virus, SARS-CoV-2, human-derived tracheal/bronchial epithelial cells*

1. Introduction

The emergence of outbreaks caused by respiratory viruses has been attracting considerable interest due to their potential to trigger pandemics such as the 1918 Spanish flu, the 2019 Coronavirus disease, and recently the Respiratory syncytial virus (RSV) in pediatric populations[1-3]. According to the World Health Organization, each year, there are an estimated one billion cases worldwide, resulting in about three to five million severe cases and 290,000 to 650,000 deaths from respiratory-related diseases[4]. The ongoing increase in cases of RSV, Influenza, and COVID-19 has triggered a critical need to identify potential chemotherapeutic agents that could be included as part of the countermeasures to aid in the preparedness for a rapid public health response in case of a pandemic[7-10].

In the present study, we have explored three sugar alcohols with potential activity against SARS-CoV-2, RSV, and Influenza, namely xylitol, sorbitol, and erythritol. Sugar alcohols, also known as polyols, are used as sweeteners and bulking agents[11]. Naturally found in many foods, sugar alcohols are derived from plant products such as fruits and berries[12]. Sugar alcohols have been known for their wide range of uses, including preventing dental cavities, bacterial infections, and they have been characterized as displaying certain antiviral activities. For example, xylitol, a sweetener, is found naturally in plums, strawberries, cauliflower, and pumpkin[13], displays antimicrobial and anti-inflammatory antiviral properties and has been shown effective in decreasing the incidence of dental caries[14-15]. In addition, xylitol helps improve chronic rhinitis, and it has immunological modulatory effects. Interestingly, sorbitol has been identified as an agent with effective antiviral action against Ebola [21]. The above-mentioned sugar alcohols have a very favorable safety profile and are used extensively in the food and drug industry, and could be manufactured and distributed widely.

Accordingly, the aim of the present *in vitro* analysis was to examine the antiviral properties of xylitol, sorbitol, and erythritol against SARS-CoV-2, RSV, and Influenza using via a highly differentiated, three-dimensional (3-D), in vitro model of normal, intact, human-derived tracheal/bronchial epithelial (TBE) cells as a preferred translational approach than the typically used Vero cells.

2. Materials and Methods

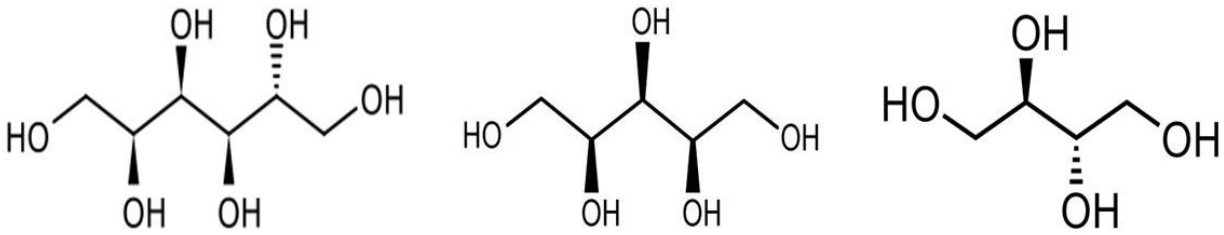
The antiviral activity of three sugar alcohols commonly utilized in the food and pharmaceutical industry, namely sorbitol, erythritol, and xylitol, were evaluated against Influenza (H1N1), Respiratory Syncytial Virus (RSV: A2), and SARS-CoV-2 (B.1.617.2; Delta) via a highly differentiated 3-D, in vitro model of normal, intact, human-derived tracheal/bronchial epithelial (TBE) cells. The sugar alcohol solutions were tested at a 5% concentration, a typically used concentration in various products and studies, in duplicate inserts of the 3D tissue models of the human airway[15 22]. Antiviral activity was measured in virus yield reduction assays by calculating the log reduction value (LRV) defined as the mean reduction of virus compared to the mean virus

control on day 3 (H1N1), day 5 (RSV), and day 6 (SARS- CoV-2) after infection. Antiviral agents utilized as comparators were Ribavirin (H1N1, RSV) and Remdesivir (SARS-CoV-2).

2.1 Compounds

The compounds received as solids were dissolved, broth to solutions, added in the MatTek culture medium (AIR-100-MM), and further diluted to the test dilutions. In the culture medium, sorbitol (45%) was received in solution and further diluted to 5% test dilutions. Ribavirin (ICN Pharmaceuticals, Inc. Costa Mesa, CA) or Remdesivir (MedChemExpress, cat# HY-104077) were tested as positive controls.

Figure 1. Molecular Structures of the tested sugar alcohols



2.2 Cell Culture

The EpiAirway™ is a validated model that consists of normal, human-derived tracheal/bronchial epithelial (TBE) cells cultured to form a multi-layered, highly differentiated model closely resembling the epithelial tissue of the respiratory tract[22 23]. The cell cultures were made to order by MatTek Life Sciences (https://www.mattek.com) (Ashland, MA) and arrived in kits with either 12- or 24-well inserts each. The TBE cells were grown on 6mm mesh disks in transwell inserts. During transportation, the tissues were stabilized on a sheet of agarose, which was removed upon receipt. One insert was estimated to consist of approximately 1.2 x 10^6 cells. Kits of cell inserts (EpiAirway™ AIR-100, AIR-112) originated from a single, healthy, non-smoker donor #9831, which was ethically obtained per the Research Involving Human Biological Materials: Ethical Issues and Policy Guidance, National Bioethics Advisory Commission, USA. Upon arrival, the cell transwell inserts were immediately transferred to individual wells of a 6-well plate according to the manufacturer's instructions, and 1 mL of MatTek's proprietary culture medium (AIR-100- MM) was added to the basolateral side.

In contrast, the apical side was exposed to a humidified 5% CO_2 environment. The TBE cells were cultured at 37°C for a minimum of one day before the experiment's performance. After the equilibration period, the mucin layer, secreted from the apical

side of the cells, was removed by washing with 400 µL pre- warmed 30 mM HEPES buffered saline solution 3X. The culture medium was replenished to the basal side following the wash steps. The tissues were then allowed to rest in a 37°C and 5% CO_2 environment for a minimum of 1 hour prior to the assay. The virus stocks were diluted in AIR-100-MM and infected at MOI 0.01 (H1N1),MOI 0.01 (RSV) and MOI 0.02 (SARS-CoV-2) CCID50 per cell, respectively.

2.3 Experimental design

Each compound treatment (140 µL) is applied to the apical side and culture, medium only is applied to the basal side (1 mL), for a 2-hour incubation. The virus was added (140 µL) to the apical side for a 2-hour infection period. As a virus control, some cells were treated with a placebo (cell culture medium only). Following the infection, the apical medium was removed, wells are washed once with media, and fresh test compoundis added to the apical side. The basal side was replaced with fresh medium. The cells were maintained at the air-liquid interface. On days 3 (H1N1), 5 (RSV), or 6 (SARS-CoV-2) post-infection, the medium was removed and discarded from the basal side. The virus released into the apical compartment of the TBE cells was harvested by the addition of 400 µL of culture medium that was pre-warmed at 37° C. The contents were incubated for 30 min, mixed well, collected, thoroughly vortexed, and plated on MDCK (H1N1), MA-104 cells (RSV), or Vero E6 cells (SARS-CoV-2) for VYR titration. Triplicate wells were used for virus control.

2.4 Determination of virus titers from each treated cell culture

MDCK (H1N1), MA-104 cells (RSV), or Vero E6 cells (SARS-CoV-2) cells were seeded in 96-well plates and grown overnight (37°C) to confluence. Samples containing the virus were diluted in 10-fold increments in the infection medium and 200 µL of each dilution was transferred into respective wells of a 96- well microtiter plate. Four microwells were used for each dilution to determine 50% viral endpoints. After 3-7 days of incubation, each well scored positive for the virus if any cytopathic effect (CPE) was observed compared with the uninfected control. The Reed-Muench method calculated the virus dose that could infect 50% of the cell cultures (CCID50 per 0.2 mL)[23].

3. Results:

The log reduction value (LRV) was calculated as the average reduction of virus compared to the average virus control on Influenza H1N1, RSV, and SAR-CoV-2 when incubated with a single concentration of each sugar alcohol (erythritol, xylitol, and sorbitol). Erythritol displayed the most antiviral efficacy against H1N1 with an LRV of 3.17 compared to the virus control LRV. Erythritol was effective at reducing >3 log10 CCID50 infectious virus from, 7.5 log10 CCID50/0.2 mL to an undetectable amount of infectious virus (Table 1).

Table 1. Antiviral efficacy against Influenza A/CA/07/09 (H1N1)

Test Compounds	Concentration (%)	[a]Log_{10} $CCID_{50}$ virus per 0.2 mL	[b]LRV
Sorbitol	5 5	6.30 5.30	1.47
Erythritol	5 5	3.50 4.67	3.17*
Xylitol	5 5	6.00 6.00	1.27
Ribavirin	100 µg/ml 10 1	0.67 5.50 7.30	[c]EC90 3.1 SI >32
Virus Control Influenza A/CA/07/09 (H1N1)	MOI 0.01	7.30 7.00 7.50	Avg. 7.27

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.

[a]Titer results from the virus yield reduction assay.

[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control

[c]EC90 = 90% effective concentration (reduce virus yield by 1 log10) as determined by regression analysis.

*Effective against the tested virus.

Both sorbitol and xylitol effectively inactivated RSV with an LRV of 2.49 and 2.65, respectively. Both sorbitol and xylitol effectively reduced>3 log10 CCID50 infectious virus from, 4.5 log10 CCID50/0.2 mL to a median of 1.83 (Table 2).

Table 2. Antiviral efficacy against Respiratory Syncytial Virus (RSV) strain A2

Test Compounds	Concentration (%)	[a]Log_{10} $CCID_{50}$ virus per 0.2 mL	[b]LRV
Sorbitol	5 5	2.00 2.00	2.49*
Erythritol	5 5	3.00 2.30	1.84
Xylitol	5 5	2.00 1.67	2.65*
Ribavirin	100 µg/ml 10 1	1.30 3.30 4.67	[c]EC90 4.6 SI >22
Virus Control RSV A2 ATCC VR-1540	MOI 0.01	4.50 4.67 4.30	Avg. 4.49

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.

[a]Titer results from the virus yield reduction assay.

[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control

[c]EC90 = 90% effective concentration (reduce virus yield by 1 log10) as determined by regression analysis.

*Effective against the tested virus.

All tested sugar alcohols (sorbitol, erythritol, and xylitol) were able to inactivate SARS-CoV-2 Delta with a median LRV of 3.50 (Table 3).

Table 3. Antiviral efficacy against SARS-CoV-2 strain USA/PHC658/2021 (B.1.617.2;delta).

Test Compounds	Concentration (%)	[a]Log_{10} $CCID_{50}$ virus per 0.2 mL	[b]LRV
Sorbitol	5 5	1.50 1.67	3.50*
Erythritol	5 5	1.67 2.00	3.29*
Xylitol	5 5	1.50 1.00	3.84*
Remdesivir	5 µM 0.5 0.05	0.67 2.50 5.00	[c]EC90 0.12 SI >42
Virus Control SARS-CoV-2 USA /PHC658 /2021 (B.1.617.2; delta)	MOI 0.02	5.30 5.30 4.67	Avg. 5.09

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.

[a]Titer results from the virus yield reduction assay.

[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control

[c]EC90 = 90% effective concentration (reduce virus yield by 1 log10) as determined by regression analysis.

*Effective against the tested virus.

4.0 Discussion

The present study sought to examine the potential *in vitro* antiviral effects of three sugar alcohols erythritol, sorbitol, and xylitol. The results of the present study reveal that all three sugar alcohols displayed virucidal activity against at least 2 of the tested pandemic-inducing respiratory viruses. Specifically, erythritol showed antiviral activity against H1N1, sorbitol and Xylitol against RSV, and all three-sugar alcohol displayed antiviral activity against SARS-CoV-2. The present results provide early evidence to encourage future pilot or clinical studies aimed at investigating the use of sugar alcohols as a potential countermeasure against respiratory viruses.

Preliminary data from our group suggests that xylitol displays weak antiretroviral activity and viral blocking properties. Mechanistically, regarding the viral blocking action of xylitol, it seems that D-xylose is the initiating element for sulfated glycosaminoglycans (GAG) that attach to the core protein[24 25]. D-xylose can be derived from xylitol by d-xylose reductase action replenishing this carbohydrate that is

targeted by the SARS-CoV-2 virus. If the virus attaches to the d-xylose position on the GAG, such as heparin sulfate, the virus can then contact the ACE2 receptor. Additionally, based on prior research, we speculate the virus adsorption is impaired due to D-xylose (xylitol) production of glycosaminoglycans decoy targets and hence may be blocking viral adsorption. Elucidation of the precise mechanism of action of the three sugar alcohols is beyond this work's scope. The authors recognize that further research into the mechanism and the cellular compartment in which sugar alcohols combine with cells to prevent viral absorption penetration and replication is essential to understand these compounds' potential better and should be prioritized.

As with any research study, the present experimental design is not free from limitations. In this study, we did not assess the relevance of the time-dependent effect of the sugar alcohol effect *in vivo*, it will be important to verify if adding the spray-on cells previously infected at nontoxic doses would exert a reduction of the viral titer. Also, whether pre-treating the cells and subsequently adding the virus would decrease the infection rate would be needed to assess the possible preventive use of sugar alcohol as a chemotherapeutic countermeasure agent. Also some samples were studied only in duplicates. Another limitation is that *in vitro* studies cannot be easily extrapolated to humans. However, recent clinical studies have documented promising results suggesting that intranasally administered xylitol may be beneficial in decreasing nasal viral loads and preventing SARS-CoV-2-induced persistent anosmia[26-29]. Moreover, it is known that many respiratory viruses use the intranasal route as a primary portal of entry, and hence has been proposed as a preferred route of administration for potential countermeasures against respiratory viruses[30-32]. However, further studies to assess the feasibility of using the intranasal route as the preferential means of administering antiviral agents warrant further experimentation.

5. Conclusion

This study demonstrates the solid antiviral potential against H1N1, RSV, and SAR-CoV-2 of the sugar alcohols xylitol, sorbitol, and erythritol. These data suggest that sugar alcohols can inactivate respiratory viruses with pandemic potential if taken together. Using sugar alcohol formulations for nasal spray applications could become a significant element in preventing and treating respiratory viruses, especially in nations where the healthcare system would be dangerously compromised by adopting less effective and significantly more financially demanding therapies. Because sugar alcohols have a favorable safety profile, they can be available over the counter without a prescription. However, additional research is needed to advance the development of sugar alcohols as countermeasures against pandemic respiratory viruses. To further ascertain the impact of sugar alcohols as countermeasure agents, we propose performing a randomized placebo-controlled study of intranasally delivered sugar alcohol in patients with a

respiratory virus and randomized placebo-controlled preventive trials in at risk populations such as healthcare workers.

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