EFFICACY OF BIOFLAVONOIDS OF FLAVOBAC™ AGAINST SEVERE ACUTE RESPIRATORY SYNDROME-CORONAVIRUS 2 IN VITRO

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ABSTRACT

Introduction The Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) is responsible for the global pandemic of Coronavirus disease-2019 (COVID-19). Human-to-human transmission occurs mainly through the aerosolization of respiratory droplets. Improved antisepsis of human and non-human surfaces has been identified as a key feature of transmission reduction. Flavobac, a complex of soluble bioflavonoids and hydroxylated phenolic structures used in oral care products, has demonstrated efficacy to act against microorganisms. This study evaluated nasal and oral antiseptic formulations of FLAVOBAC for the virucidal activity against SARS-CoV-2.

Methodology FLAVOBAC nasal antiseptic formulations and FLAVOBAC oral rinse antiseptic formulations from 1-10% concentrations were assayed for virucidal efficacy against the SARS-CoV-2 virus. SARS-CoV-2 was exposed directly to the test compound for 60 seconds or 5 minutes. Compounds were then neutralized, and the surviving virus was quantified.

Results All concentrations of nasal antiseptics and oral rinse antiseptics evaluated completely inactivated the SARS-CoV-2 virus.

Conclusion Nasal and oral FLAVOBAC solutions are effective at inactivating the SARS-CoV-2 virus at a variety of concentrations after 60-second or 5-minute exposure times. The formulations tested have the potential to reduce the transmission of SARS-CoV-2 if used for nasal/oral decontamination, or surface decontamination in known or suspected cases of COVID-19.

KEYWORDS

Bioflavonoid; COVID-19; Flavobac, SARS-COV-2; Virucidal Activity.

1. INTRODUCTION

The emergence of the novel human Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) has caused drastic damage to the globe with a dramatic loss of human life worldwide. To date (4 June 2021), approximately 172 million cases have been confirmed with over 3.7 million deaths according to WHO [1]. Previous studies confirmed that viral loads are high in the nasal cavity, nasopharynx, and oropharynx [2-5]. SARS-CoV-2 viral RNA levels (measured in saliva, throat and nasal swabs) are highest at the time of, soon after, or before symptoms and SARS-CoV-2 RNA are detected in saliva and throat swab specimens in recovered patients 5 to >40 days following hospital discharge [6]. Furthermore, live SARS-CoV-2 was isolated from throat swab specimens, indicating viral replication and shedding are active in tissues of the upper respiratory tract where SARS-CoV-2 is not thought to replicate [7]. SARS-CoV-2 appears to remain viable while suspended in aerosols for ~3h and on surfaces for days possibly indicating disease spread might occur even at considerable distances and in enclosed spaces with poor ventilation [8].
Furthermore, among genetically related coronaviruses, SARS-CoV-2 is predicted to have the hardest protective outer shell against degradative enzymes thus contributing to its high resilience in saliva, other body fluids, and outside the body [9]. Despite many clinical trials that have been conducted for the treatment of COVID-19, no antiviral has been verified to be effective for COVID-19. Conventional antiviral drugs including ribavirin and favipiravir and other anti-inflammatory agents are currently used in clinical settings against the severe cases of SARS-CoV-2 infections [10]. On the other hand, increased interests in plant-based natural products belonging to the flavonoids class have emerged as an attractive option of treatment. The low cytotoxicity and synergy with other effective drugs make flavonoids an ideal candidate to interfere with the life cycle of the virus. Several flavonoids have been shown to exhibit significant antiviral properties in in vitro and in vivo studies [11-13]. Bioflavonoids are naturally present in plants, fruit and vegetables, there are over 6000 identified having a wide range of uses including antioxidants & food ingredients. Flavonoids comprise a group of naturally occurring compounds that are among the most ubiquitous in the plant kingdom [14]. They are found in every family and nearly every species of the higher plants and they have extensive biological properties that promote human health and help reduce the risk of diseases. Bioflavonoids have demonstrated their capacity to act against bacteria, fungi, and viruses [12,15,16]. Flavobac (Trademark of Citrox Bioscience Ltd) is a natural product extracted from bitter oranges and composed of soluble bioflavonoids and hydroxylated phenolic structures. Flavobac is developed as nasal and oral rinse antiseptic containing Flavobac BCL concentrate as the sole active ingredient and OSI-20210203 oropharyngeal spray solution consisting of aqueous Flavobac BCL 2% (A) and 5% (B) and other natural ingredients were supplied by Oral Science International (Montreal, Canada).

In the current study, we hypothesized that flavonoids as active ingredients in the Flavobac may have virucidal activities against SARS-CoV-2 which may enable a new application for protection against COVID-19. We report for the first time the virucidal activity of commercially available 2-5% Flavobac solutions against the human novel SARS-CoV-2 strain SARS-CoV-2, USA-WA1/2020. We report here the first anti-SARS-CoV-2 evaluation of a nasal and oral rinse antiseptic containing Flavobac which has been developed specifically for routine intranasal or oral use.

2. METHODS

2.1. Virus culture and biosafety and test compounds

SARS-CoV-2, USA-WA1/2020 strain obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA), was used in this study for the virucidal activity of Flavobac. The virus stock was prepared by passaging three times in Vero 76 (ATCC, CRL-1587) cells. The media was MEM supplemented with 2% fetal bovine serum (FBS, Cytivia) and 50 μg/mL gentamicin (Sigma). Viral cultures were carried out in a biosafety level 3 (BSL-3) following standard operating procedures approved by the USU Biohazards Committee, Institute for Antiviral Research, Utah State University, USA. Nasal antiseptic solutions and oral rinse antiseptic solutions consisting of aqueous Flavobac BCL concentrate as the sole active ingredient and OSI-20210203 oropharyngeal spray solution consisting of aqueous Flavobac BCL 2% (A) and 5% (B) and other natural ingredients were supplied by Oral Science International (Montreal, Canada).

2.2. Virucidal Assay

Flavobac BCL concentrate was diluted to 10%, 5%, 2%, or 1% in the test media and OSI-20210203 samples were tested at full strength. Prepared test compounds were mixed with the virus stock solution (approximately 5.5 log_{10} CCID50 per 0.1 mL) at a volume ratio of 9:1 (v/v). Each concentration was tested in triplicate. The Test media was added to one tube of each prepared concentration to serve as toxicity controls. Water was tested in parallel as a negative control. The prepared test compound and the virus solution mixtures were incubated at room temperature for 1 min, 5 min (approximately 5.5 log_{10} CCID50 per 0.1 mL) at a volume ratio of 9:1 (v/v). Each concentration was tested in triplicate. The Test media was added to one tube of each prepared concentration to serve as toxicity controls. Water was tested in parallel as a negative control.

In the current study, we hypothesized that flavonoids as active ingredients in the Flavobac may have virucidal activities against SARS-CoV-2 which may enable a new application for protection against COVID-19. We report for the first time the virucidal activity of commercially available 2-5% Flavobac solutions against the human novel SARS-CoV-2 strain SARS-CoV-2, USA-WA1/2020. We report here the first anti-SARS-CoV-2 evaluation of a nasal and oral rinse antiseptic containing Flavobac which has been developed specifically for routine intranasal or oral use.

Table 1. Virucidal efficacy against SARS-CoV-2 after contact with the virus at 22 ± 2°C.

| Compound          | Contact Time | Cytotoxicity | Neut. Ctrl. | Virus Titer | Virus Titer | LRV |
|-------------------|--------------|--------------|-------------|-------------|-------------|-----|
| FLAVOBAC BCL 10%  | 1 min        | 1/100        | None        | <2.7        | 4.6         | >1.9|
| FLAVOBAC BCL 5%   | 1 min        | 1/100        | None        | <2.7        | 4.6         | >1.9|
| FLAVOBAC BCL 2%   | 1 min        | 1/10n        | None        | <1.7        | 14.6        | >2.9|
| FLAVOBAC BCL 1%   | 1 min        | 1/10         | None        | <1.7        | 4.6         | >2.9|
| OSI-20210203A     | 5 min        | 1/10         | None        | <1.7        | 4.7         | >3.0|
| OSI-20210203B     | 5 min        | 1/10         | None        | <1.7        | 4.7         | >3.0|

1 Cytotoxicity indicates the highest dilution of the endpoint titer where full (80-100%) cytotoxicity was observed.
2 Neutralization control indicates the highest dilution of the endpoint titer where compound inhibited virus CPE in the wells after neutralization
3 Virus titers of the test sample or virus control (VC) in log_{10} CCID50 of virus per 0.1 mL
4 LRV (log reduction value) is the reduction of virus in the test sample compared to the virus control.
temperature (22 ± 2°C) for 1 or 5 minutes as indicated in Tab. 1. The assay was neutralized by a 1/10 dilution in MEM 2% FBS, 50 μg/mL gentamicin. For quantification, the surviving virus from each sample was quantified by standard end-point dilution assay. Briefly, the neutralized samples were pooled and serially diluted using eight log_{10} dilutions in a test medium. Then 100 μL of each dilution was plated into quadruplicate wells of 96-well plates containing 80-90% confluent Vero E6 cells (ATCC CRL-1586). The toxicity controls were added to an additional 4 wells of Vero E6 cells and 2 of those wells at each dilution were infected with the virus to serve as neutralization controls, ensuring that residual sample in the titer assay plate did not inhibit growth and detection of the surviving virus. Plates were incubated at 37 ± 2°C with 5% CO2 for 5 days. Each well was then scored for the presence or absence of an infectious virus. The titers were determined using the Reed-Muench (1948) equation [17] and the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.

3. RESULTS

Flavobac BCL aqueous solution showed to have potent antiviral activities by reducing SARS-CoV-2 viral titer below the limit of detection (LoD) at all concentrations tested in vitro. We have tested various concentrations to ensure that we identify the optimal concentration of Flavobac BCL against SARS-CoV-2. Flavobac BCL is a key ingredient of the formulation OSI-20210203. The latter is the commercialized product (Cold & Flu Guard). Because of differences in cytotoxicity, the lower concentrations had higher LRV values with LRV>2.9 at 2% and 1% and LRV>1.9 at 5% and 10%, respectively (Table 1). Furthermore, Virus titers and LRV of SARS-CoV-2 after incubation with Flavobac commercial solutions OSI-20210203A and OSI-20210203B reduced the virus below the limit of detection (LRV>3.0). Each Flavobac containing solution evaluated was effective at reducing >3 log_{10} CCID50 infectious virus, from 4.7 log_{10} CCID50/0.1 mL to <1.7 log_{10} CCID50/0.1 mL. A consistent reduction in virus titre was observed for SARS-CoV-2 viruses at all concentrations of Flavobac solution tested and with a contact time of 1 or 5 minutes. The reductions in virus titre were >1.9 log_{10} CCID50/mL for Flavobac 5 and 10%, equating to >98.7% reduction; >2.9 log_{10} CCID50/mL for Flavobac 1 and 2%, equating to >99.8% reduction; and >3.0 log_{10} CCID50/mL, equating to >99.9% reduction for OSI-202103, the commercial formulation of Flavobac.

4. DISCUSSION

In this era of emerging infectious diseases caused by respiratory tract viruses especially SARS-CoV-2, finding new alternative biomedical tools based on natural products is important to suppress viral activities to pre-pandemic level, the heightened fear of a post-pandemic outbreak looms as the virus still lurks and relatively little is known about the key parameters that could shape the future course of the pandemic [25].
The challenge in nasal antisepsis is to find effective topical preparations which are safe to use. Oral and nasal solutions infused with Flavobac, a natural bioflavonoid complex, with broad virucidal efficacy, has demonstrated efficacy to kill 99.9% when incubated with the virus for 1 to 5 minutes at 22°C. The results indicate that Flavobac oral and nasal spray could potentially be used to neutralize SARS-CoV-2 and reduce viral transmission.

5. CONCLUSION

Current public health recommendations emphasize the need to break the person-to-person viral transmission primarily through social separation, hand hygiene and surface disinfection. Flavobac deactivated SARS-CoV-2 virus tested from 98.8% to > 99.9% when incubated with the virus for 1 to 5 minutes at 22°C. The results indicate that Flavobac oral and nasal spray could potentially be used to neutralize SARS-CoV-2 and reduce viral transmission.

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CONFLICT OF INTEREST

Dr. Glogauer is the Chief Scientific Officer for Oral Sciences. Oral Sciences is the developer of this rinse product. The rest of the authors declare no conflict of interest. Dr. Glogauer is the Chief Scientific Officer for Oral Sciences. Oral Sciences is the developer of this rinse product. The rest of the authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

AB, MG contributed to the concept, design, and data analysis of the study and wrote the manuscript. MM performed the experiments. GD, HL contributed to the data analysis and edited the manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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Dr. Abdelahhad Barbour is a Postdoctoral scientist and research coordinator of the COVID-19 saliva testing project at the Faculty of Dentistry, University of Toronto. The project aims to document rates of COVID-19 infection and immune responses to vaccination among trainees, faculty, and staff in Canadian dental schools. Dr. Barbour obtained his PhD in Molecular Microbiology from the University of Malaya, Malaysia with a thesis entitled “Characterisation and mechanism of action of lantibiotics produced by Streptococcus salivarius”. He is an expert in the fields of antimicrobial peptides, microbial genomics, oral microbiome and probiotics developments. Areas of interest in the research activity: Host-microbiome interactions, neutrophils-pathogens signaling, COVID-19 saliva testing, antibiotic resistance, microbial genomics.

Questions

1. What are bioflavonoids?
   a. Synthetic substance;
   b. Natural products derived from animal origin;
   c. Natural products derived from fruits and vegetables;
   d. Antibiotics produced by soil microorganisms.

2. What are the biological activities of bioflavonoids?
   a. Antimicrobial;
   b. Antiviral;
   c. Antifungal;
   d. All mentioned above.

3. What is the main composition of FLAVOBAC™?
   a. Soluble bioflavonoids and hydroxylated phenolic structures;
   b. Antimicrobial peptides;
   c. Polysaccharides;
   d. Lipids.

4. FLAVOBAC™ deactivated the SARS-CoV-2 virus at the following levels:
   a. 10-20%;
   b. Less than 50%;
   c. Less than 80%;
   d. More than 95%.