TaffiX® nasal powder spray forms an effective barrier against infectious new variants of SARS-CoV-2 (COVID-19)

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Short Report

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Abstract

Introduction: While vaccination efforts against SARS-CoV-2 around the world are ongoing -, new high-infectious variants of the virus are being detected. The protection of the available vaccines against some of the new variants is weaker, and experts are concerned that newer as yet undescribed variants of this mutated RNA virus will eventually prove stable against the current vaccines. Additional preventive measures will therefore be needed to protect the population until effective vaccinations are widely available.

TaffiX® is a personal, anti-viral nasal powder spray comprised of low pH Hypromellose that upon insufflation into the nose creates a thin gel layer covering the nasal mucosa and forming a protective mechanical barrier that prevents viruses from engaging with nasal cells- the main portal of entry for viruses. Taffix is commercially available in many countries across Europe, Asia America and Africa. In a prior preclinical study, *TaffiX®* was found to be effective against SARS-CoV-2 Hong Kong/VM20001061/2020 in experimental in vitro conditions. A real-life clinical survey demonstrated that *TaffiX®* nasal spray significantly reduced the SARS-CoV-2 infection rate post mass-gathering event in a highly endemic community.

Objective: The current study aimed to test the protective effect of Taffix against new pathogenic, highly infectious SARS-CoV-2 variants in vitro: the “British” B.1.1.7 (hCoV-19/Israel/CVL-46879-ngs/2020) and the “South African” B.1.351 (hCoV-19/Israel/CVL-2557-ngs/2020) variants.

Study design: A TaffiX® gel was formed on a nylon filter, using an amount equivalent to a clinical dose of Taffix . Filters were then seeded with SARS-CoV-2 B.1.1.7 (“British”) and B.1.351 (“South African”) variants. After a 10 -minute incubation at room temperature, the bottom of each filter was washed, and the resulting flow-through was collected and seeded into 24 -well plates containing Vero-E6 cells. After 5 days of incubation, a 200 µl sample from each well was taken for viral RNA extraction followed by SARS-CoV 2 RT-PCR analysis.

Results: The *TaffiX®* gel completely blocked SARS-CoV-2 highly infectious variants B.1.1.7 and B.1.351 *in vitro*, reducing the titer of recoverable infectious virus as well as viral RNA by 100%.

Conclusions: Under in vitro conditions, TaffiX® formed an effective protective barrier against SARS-COV-2 variants (British variant and South African Variant). These results are consistent with prior findings demonstrating the *in vitro* high efficacy of Taffix gel in preventing viruses from reaching cells and infecting them. These results, added to clinical real-life studies performed with Taffix , support its use as an effective barrier against new variants of SARS-CoV-2 in conjunction with other protective measures.

Introduction

While vaccination efforts against SARS-CoV-2 around the world are ongoing, new high-infectious variants of the virus are being detected. The protection of the available vaccines against some of the new variants

is weaker, and experts are concerned that newer as yet undescribed variants of this rapidly mutating RNA virus will eventually prove stable against the current vaccines .Additional preventive measures will therefore be needed to protect the population until effective vaccinations are widely available.

It is now well established that the nasal epithelium, specifically goblet and cilia cells, is the primary portal of entry of the SARS-CoV 2 virus into the body, and protection of nasal cells could therefore be meaningful in reducing the risk of infection .

TaffiX® is a personal, anti-viral nasal powder spray comprised of low pH Hypromellose that upon insufflation into the nose creates a thin gel layer covering the nasal mucosa forming a protective mechanical barrier that prevents viruses from engaging with nasal cells. The acidity of the gel enhances the barrier by inactivating the viruses trapped in it. Taffix is commercially available in many countries across Europe, Asia America and Africa. In a prior pre-clinical study *TaffiX®* was found effective against SARS-CoV-2 Hong Kong/VM20001061/2020 using experimental in vitro conditions. In a real-life clinical survey *TaffiX®* nasal spray significantly reduced SARS-CoV-2 infection rate post mass-gathering event at a highly endemic community

In this study we present *in vitro* data on the protective effect of Taffix against the new pathogenic, highly infectious variants of the SARS- CoV-2 : the “British” B.1.1.7 (hCoV-19/Israel/CVL-46879-ngs/2020) and the “South African” B.1.351 (hCoV-19/Israel/CVL-2557-ngs/2020) variants.

Materials And Methods

Virus B.1.1.7 stock was isolated from patients in Israel on 20 December 2020; virus B.1.351 stock was isolated from patients in Israel on 15 January 2021; cell strainers (40 µm Cat# SO SCS402) were obtained from AlexRed; qRT-PCR is described below; cell culture media (MEM-EAGLE) was obtained from BI Beit Haemek; Vero-E6 cells were obtained from HTCC; *TaffiX®* was supplied by Nasus Pharma.

TaffiX® Assay

One hundred fifty (150) µl of sterile water was added to individual sterile 40 µm cell strainers placed on a 50 ml tube. Twenty milligrams of *TaffiX®* was added and mixed with the water with a pipet tip until homogeneous across the cell strainer surface and incubated at room temperature for 10 minutes. Control filters were incubated with 150 µl of sterile water. Filters were then seeded with 10 µl of SARS-CoV-2 British or South African variants (each variant separately) at a concentration of Cycle Threshold (CT) 16.5 (approximately 5,000,000 copies/ml) or CT 20 (approximately ~ 600,000 copies/ml), which are parallel to the clinical concentrations found in patients’ nasal swabs diagnosed positive for SARS-CoV-2 (COVID-19).

After a 10-minute incubation at room temperature, the bottom of each filter was washed with 500 µl cell culture media, and the resulting flow-through was collected and diluted 1:10 with culture media (to prevent cytotoxicity to the Verro-E6 cells due to the acidic pH of *TaffiX®*). Then, 50 µl of the diluted flow-

through was seeded into 24-well plates containing Vero-E6 cells ($2x10^5$ cells/well, in 2% fetal calf serum). After 5 days of incubation at 33°C (5% CO_2), a 200 µl sample from each well was taken for viral RNA extraction followed by qRT-PCR analysis. Each treatment was repeated twice.

Quantitative Reverse Transcriptase PCR (qRT-PCR)

Nucleic acids were extracted using the MagLead extractor (PSS, Japan) according to the manufacturer's instructions. RT-PCR reactions using primers corresponding to the SARS-CoV-2 envelope (E) gene were performed as previously described (Corman et al., Eurosurveillance, 2020) . qRT-PCRs were performed in 25 µl SensiFast reaction mix (Bioline, USA) using TaqMan Chemistry on a CFX-96 instrument.

Results

Using an amount equivalent to a clinical dose, the *TaffiX®* layer completely (100%) protected Vero-E6 cells from infection by two variants of SARS-CoV-2, B.1.1.7 (British) and B.1.351 (South African). For both variants, virus solution at an initial concentration of approximately 5,000,000 copies/ml (parallel to a clinical PCR result of CT 16.5) and 600,000 copies/ml (parallel to a clinical PCR result of CT 20) was applied to filter with and without *TaffiX®*. After 5 days of incubation of Vero- E6 cells with the flow-through solution eluted from the filter, no infection (zero) was diagnosed in the cells by qRT-PCR following the *TaffiX®* gel protection, in comparison with a control of filters without *TaffiX®*, were very high number of virus copies were detected in the cells: 2,236,550 copies/ml (filters inoculated with CT 20) and 25,027,670 copies/ml of B.1.1.7 variant (filters inoculated with CT 16.5, Fig. 1); and 506,578,602 copies/ml of the B.1.351 (filters inoculated with CT 20, Fig. 2).

In this series of experiments, the initial virus concentration used was equivalent to the clinical concentration found in nasal swabs taken from positive COVID-19 patients (CT 16.5, CT20), and they were all effectively blocked by the *TaffiX®* gel layer, which was applied at a clinical equivalent dose.

Discussion

In a prior *study, TaffiX®* nasal spray powder formed a protective barrier against SARS-COV-2 Hong Kong/VM20001061/2020 under *in vitro* conditions preventing > 99.9% of viruses from infecting cells [8]. The protective effect of *TaffiX®* in vitro was further verified in a human real-life survey conducted in Israel during September 2020 in a "superspreader" event where a reduction of 78% in COVID-19 cases was recorded among *TaffiX®* users with no reported side effects [9] .

Since then, new variants of the SARS-COV-2 infectious virus have emerged. According to the American Centers of Disease Control and Prevention (CDC) and the United Kingdom (UK) government publication , a newly identified variant called B.1.1.7 ("British") with a large number of mutations was detected in the fall of 2020. This variant spreads more easily and quickly than other variants. Recent data from the UK reported that this variant may be associated with an increased risk of death compared to other variant

viruses. It has since been detected in many countries around the world. This variant was first detected in the US at the end of December 2020 and is now the dominant variant found in new cases.

In South Africa, another variant called B.1.351 emerged independently of B.1.1.7. Originally detected in early October 2020, B.1.351 shares some mutations with B.1.1.7. Cases caused by this variant have been reported in the US at the end of January 2021

In Brazil, a variant called P.1 emerged that was first identified in travelers from Brazil who were tested during routine screening at an airport in Japan in early January 2021. This variant contains a set of additional mutations that may affect its ability to be recognized by antibodies. This variant was first detected in the US at the end of January 2021. As of March 2021, the “British” variant was the most abundant virus found in clinical samples of COVID-19 patients around the world, and the “South African” variant was the second most abundant (according to the CDC data).

Taffix is used worldwide as an additional layer of protection to help reduce the risk of contracting viral diseases caused by viruses whose first portal of entry to the body passes through the nasal cavity, such as influenza rhinoviruses and COVID-19. Its efficacy against the newly emerging variants of SARS-CoV 2 is therefore clinically relevant.

The concept of nonspecific protection against upper respiratory infectious viruses was first described. Hull *et al*. emphasized the potential value of creating a hostile microenvironment in the nasal cavity as an effective means of activating upper respiratory infectious pathogens. Indeed, many upper respiratory viruses are sensitive to low pH, including rhinoviruses , influenza , respiratory syncytial virus (RSV), and coronavirus . Hull *et al.* demonstrated in a randomized, double blind, placebo-controlled clinical study that irrigation with an acidic nasal hydrogel spray reduced the severity and duration of the common cold symptoms [19]. Sungnak et al [5 9] found that SARS-CoV-2 entry factors are highly expressed in goblet and ciliated nasal epithelial cells together with innate immune genes. These factors make the nose likely a COVID virus entry point.

TaffiX® low pH gel is formed within 1 minute from spray and an acidic film is maintained on the nasal tissue for about 5 hours. The human nasal mucosal pH is approximately 5.5–6.5 ,which is a comfortable environment for most viral pathogens including the SARS-COV-2 virus.

In this study, we have shown that *TaffiX®* is very efficient in blocking the new variants under *in vitro* conditions.

Conclusion

Under in vitro conditions, TaffiX® formed an highly effective protective barrier against SARS-COV-2 variants (British variant and South African Variant). These results are consistent with prior findings demonstrating the *in vitro* high efficacy of Taffix gel in preventing viruses from reaching cells and infecting them. The body of work, in addition to clinical real-life studies performed with Taffix, support the

use of nasal spray as an effective barrier against new variants of SARS-CoV-2 in conjunction with other protective measures.,

Since the development and distribution of new vaccines against novel variants is a long and complex process, additional protective measures are required. Taffix may provide a simple and safe way to reduce infections and thus the spread of COVID-19.

Declarations

Funding Statement

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Declaration of Competing Interest

T. Lapidot, PhD and D. Megiddo, MD are employees of Nasus Pharma.

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Figures

Figure 1: Taffix prevented infection of Vero-E6 cells in "British" variant

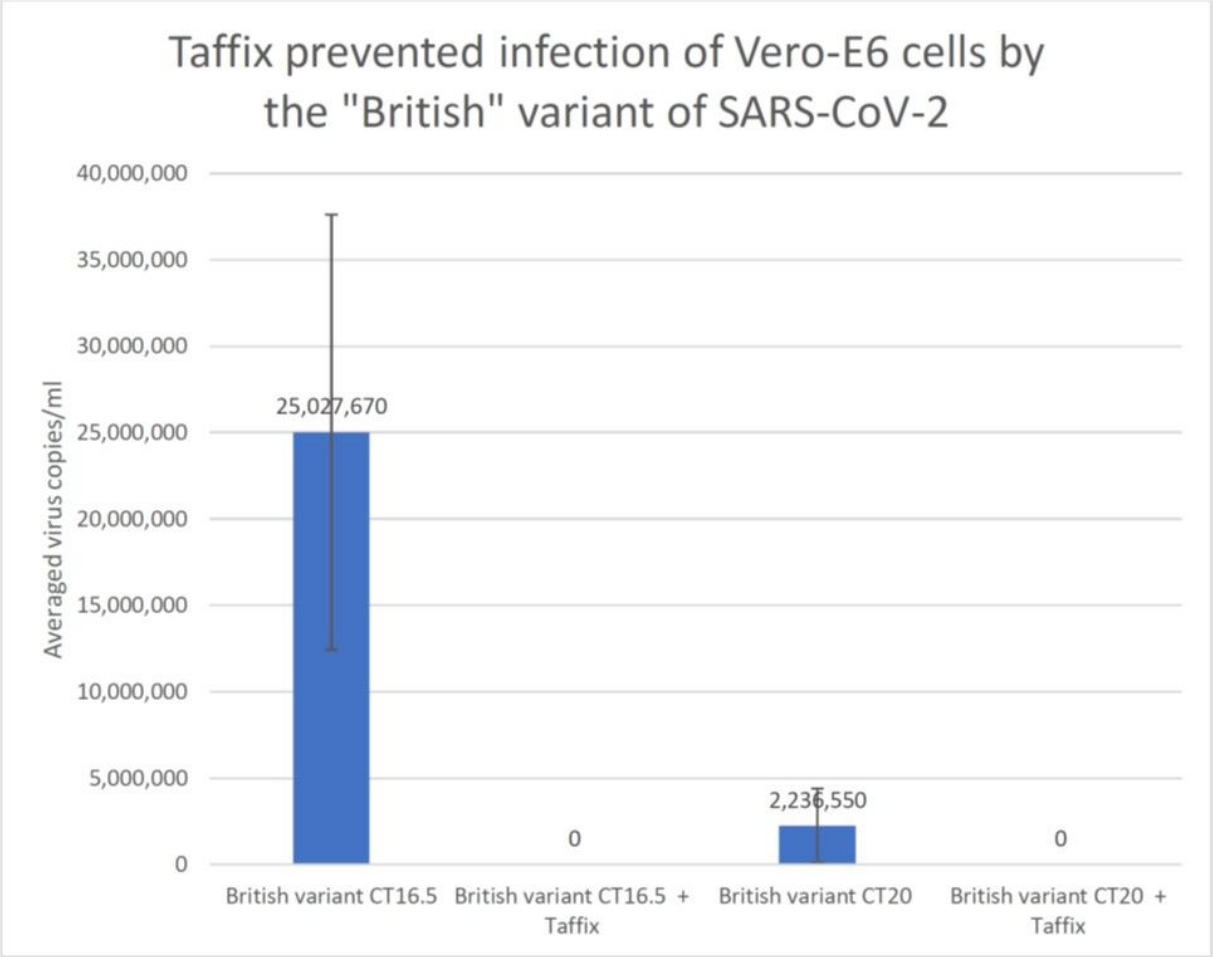

Figure 1

Vero-E6 cells were incubated with inoculum of SARS-CoV-2 variants B.1.1.7 (1) at a concentration of CT 16.5 or CT20, which had been seeded on cell strainer filters covered with Taffix (+Taffix) or without Taffix. Total RNA was extracted from the growth medium five days later for qPCR analysis. Viral copies were calculated from qPCR CT values. For each test, a positive sRNA control and negative control were used.

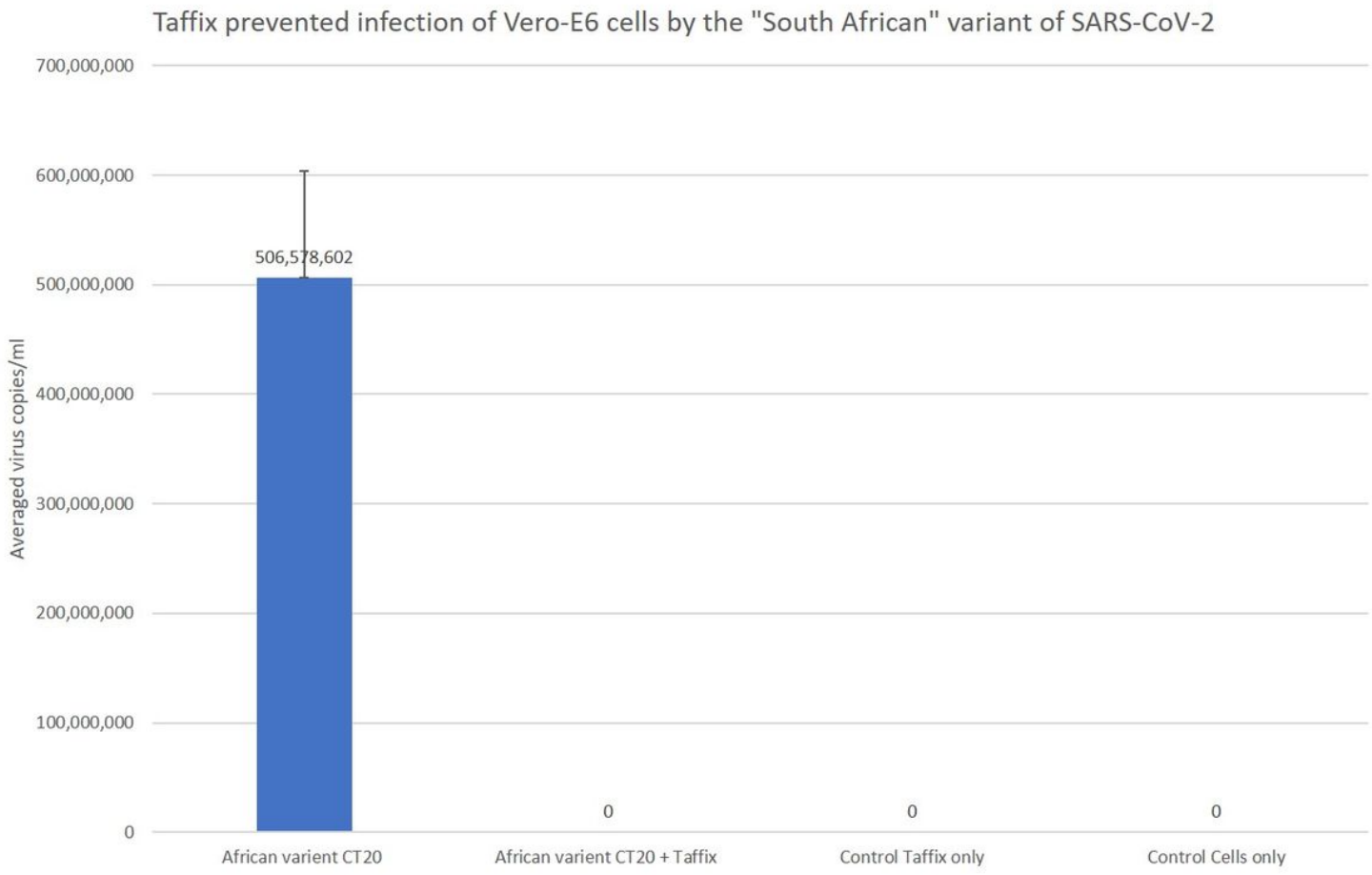

Figure 2

Vero-E6 cells were grown and incubated with inoculum of SARS- B.1.351 (2) at a concentration of CT 16.5 or CT20, which had been seeded on cell strainer filters covered with Taffix (+Taffix) or without Taffix. Total RNA was extracted from the growth medium five days later for qPCR analysis. Viral copies were calculated from qPCR CT values. For each test, a positive sRNA control and negative control were used.