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Research Article

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Decrease in Covid-19 Contagiousness: Virucidals Control the Presence of Covid in Saliva and Salivary Glands

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Abstract

Summary: *This pilot study evaluated the effects of some virucidals in vivo (using the RT-PCR swab test) to evaluate the presence of COVID in the mouth saliva. With this model, if an asymptomatic subject is positive (in the mouth saliva, the first incubating medium), virucidals can be used, i.e., for a week, and the positivity re-evaluated to define the direct killing power of the virucidal.*

Results: *A progressive decrease in positivity in the swab samples was observed. Virucidals produced disappearance of positivity in most subjects at 3 and 7 days. All virucidals used in this pilot registry resulted effective. The pharmaceutical form seems to be important to assure persisting traces of the virucidal in the mouth. The gummy Phyrorelief CC possibly produced the longest action (>3 hours) due to its slower release. These preliminary observations indicate a significant effect of virucidals on the viral contamination of the mouth (the first incubator) with a relatively simple, cost-effective model. The lower presence of a virus charge or its decrease may significantly reduce contagiousness of most of these patients and possibly, the spread of viral material. The effects on the evolution of COVID-19 on single patients is, unpredictable with this model but virucidals may assume a significant community value in preventing and controlling spreading.*

Keywords: Covid, Virucidals, Virus, Salivary Glands Virosis, Saliva, Contagion

Introduction

Covid swabs detect the presence of COVID viral elements in saliva, in vivo. The test is relatively simple and fast but not completely reliable from a diagnostic point of view (one patient can be negative today and positive tomorrow). COVID-19 testing can identify the SARS-CoV-2 virus and includes methods that detect the presence of viruses (RT-PCR, isothermal nucleic acid amplification, antigen) or antibodies. The real-time reverse transcription polymerase chain reaction (rRT-PCR) test can be done on respiratory samples (including a nasopharyngeal swab or saliva/sputum samples) [1-3]. Results are generally available within hours. The presence of viruses in saliva and (later, in mucus) is a significant source of contagion; the oropharyngeal cavity is considered the real incubator and the viruses multiply until they reach a critical mass to spread to target organs (trachea, bronchial tree and lungs). The salivary glands may also host the virus and operate as a secondary incubator; this may be linked to super spreading with prolonged release of viruses and sustained contagiousness.

This pilot study evaluated the effects of some virucidals *in vivo (*using the RT-PCR swab test) to evaluate the presence of COVID in the mouth saliva. With this model, if an asymptomatic subject is positive (in the mouth saliva, the first incubating medium), virucidals can be used, i.e., for a week, and the positivity re-evaluated to define the direct killing power of the virucidal.

Presence of Covid in Salival Glands

Viral elements in saliva – the main incubator – is associated to super spreading. In most of these subjects Covid-19 symptoms may be very mild (i.e., submandibular pain, swelling at the parotid and submandibular glands, sublingual glands, dry mouth, difficult swallowing). Salivary gland virosis may be very persisting for example with cytomegalovirus infection.

Methods, Subjects

We identified groups of asymptomatic 'patients' (found as positive) who were otherwise healthy. Their age was <55. No drugs had been used, a normal oral hygiene was regularly observed, their temperature was normal. The test was made before 10 am, without washing mouth or teeth and without breakfast. The same tests were repeated at 3 and 7 days under similar conditions. No specific drugs were used during the observation period and no significant symptoms occurred or were reported. All subjects remained completely asymptomatic. Progression to symptoms and to need for management or hospitalization for COVID-19 (or any other condition) was considered an exclusion item. All subjects respected the anti-viral measures (mask, hygiene, distancing) and used Vitamin C and a normal diet. Virucidals that are generally available without

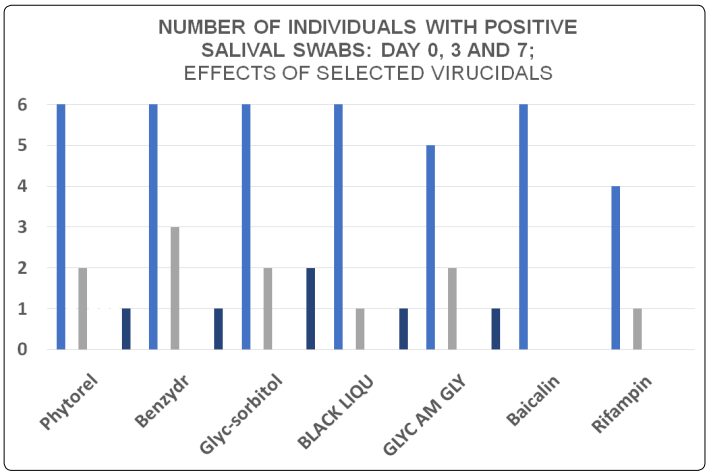
prescriptions in the pharmacy were used in the study. Products, commonly available as Over the Counter (OTC) were used. The specific selection was left to the single subjects. Rifampin is an antibiotic and formally requires a prescription (but in the situation it was used at a very low lose, < 30 mg, in a saline mouthwash).

Phytorelief, Alchem, (Germany) includes ginger, turmeric and pomegranate extracts. The gummy caramel is slowly dissolved in the mouth; its effects may last 2-3 hours. The product includes pomegranate and licorice extract that are considered both virucidals and anti-virals (if used for systemic administration).

Benzydamine (also known as Tantum Green, branded in some countries as Difflam or Septabene), available as the hydrochloride salt is a locally acting nonsteroidal anti-inflammatory drug (NSAID) with local anesthetic and analgesic properties for pain relief and anti-inflammatory treatment of mild conditions of the mouth/upper throat.

Benzydamine Cl: (Iodosan) +cetilpiridine is also a combination of oral disinfectants and an anti-inflammatory agent. Effects, possibly, do not persist for more than 2 hours after mouth washing. At the moment, there are only 2 subjects in this group. Therefore, results are not shown in (**Figure 1**).

Figure 1: the decrease in positivity in the swab samples. The use of 'virucidals' resulted in a significant level of complete disappearance of positivity in most subjects.



All virucidals were effective.

The gummy Phyrorelief CC produced the longest action due to a slower release of its components. These preliminary observations indicate a significant effect of virucidals on the viral contamination of the mouth with a relatively simple, low cost model. The lower presence of a virus charge or its decrease may significantly reduce contagiousness of most of these patients and possibly the spread of viral material.

A new compound used for dry mouth (Certmedica) shows persisting effects in the mouth (up to 3 hours). Included in a jelly liquid with glycerin, sorbitol, xylitol, aloe, polyacrylate, xanthan gum; also, lactoferrin is parts of this product. A Black liquorice extract (produced by LEPR, India) can be used either in a mouthwash and in vaporized form (using a warm-humid vaporization system). Glycamil Ammonium Glycyrrhizinate marketed by HAD, Italy) is a soluble, white liquorice preparation also usable as a mouthwash and by vaporization. Liquorice preparation may last in the mouth for at least 2 hours. Two other products that can also be used as drugs were used in a mouthwash or by vaporization by WHV: Baicalin 85% (obtained by InXi, Shanghai). This product (alone or in combinations) was diffusely used in Wuhan during the epidemic (however, data are not accessible). It is a flavone glycoside (the glucuronide of baicalein). This product is an inhibitor of 5- and platelet 12-lipoxygenases (IC50 values are 9.5 and 0.12 μM respectively). Also inhibits Raf-mediated MEK-1 phosphorylation in glioma cells and induces G1 and G2 cell cycle arrest by decreasing cdk1, cdk2, cyclin D2 and cyclin expression; it inhibits production of inflammatory cytokines by inhibiting NF-κB activation. Also inhibits erastin-induced ferroptosis.

Rifampin is an antibiotic used to treat several types of bacterial infections, including tuberculosis, Mycobacterium avium complex, leprosy, and Legionnaires' disease. It is generally used with other antibiotics, except when given to prevent Haemophilus influenzae type b and meningococcal disease in people exposed to those bacteria. A significant antiviral activity of Rifampin has been described [4-12].

This registry study evaluated in a simple, low-cost *in vivo* model the effects of these 'virucidals' on the local presence in the mouth 'incubator' (saliva) of the COVID virus. As indicated, these subjects were asymptomatic or with very minor, transient symptoms (sore throat, infrequent cough and mild fatigue). A 'virucidal' mouthwash had been suggested – 3-4 times, daily - to relieve the minor symptoms. After an initial swab (day zero) the tests were repeated at day 3 and seven.

Results

No side effects were observed. Figure 1 shows the decrease in positivity in the swab samples. The use of 'virucidals' resulted in a significant level of complete disappearance of positivity in most subjects at 3 and 7 days. The test was considered only as positive or negative without quantification of the level of positivity. All virucidals used in this pilot registry resulted effective. However, the pharmaceutical form seems to be important to assure persisting traces of the virucidal in the mouth. The gummy Phyrorelief CC possibly produced the longest action (>3 hours) due to its slower release. These preliminary observations indicate a significant effect of virucidals on the viral contamination of the mouth with a relatively simple, cost-effective model. The lower presence of a virus charge or its decrease may significantly reduce contagiousness of most of these patients and possibly, the spread of viral material. The effects on the evolution of COVID-19 on single patients is, at the moment, unpredictable with this model but virucidals may assume a significant community value in preventing and controlling spreading.

Comments-Discussion

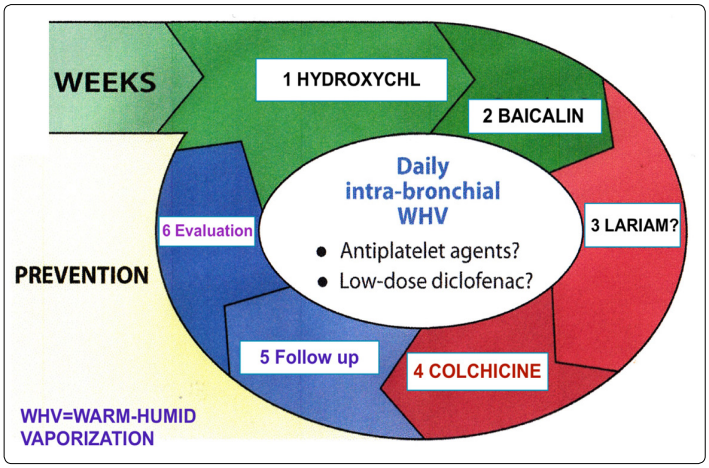
Antiviral drugs are the key product used for treating viral infections. Most antivirals target specific viruses; however, some broad-spectrum antivirals may be effective against a wide range of viruses. Antivirals do not directly destroy their target virus but, generally, inhibit their development. At the moment there are no specific antivirals, strongly active against the Wuhan viruses. Virucides or viricides, generally, are not drugs but deactivate or destroy many

viruses inside or outside the body and specifically in 'border' anatomical areas (mouth, nose) when it is possible to apply them without causing damages or side effects. Some of the most common virucidals are produced from natural plant extracts (eucalyptus and Australian tea trees) [13]. Liquorice extracts also have a significant antiviral (by systemic administration) and virucidal (local) activity. Eucalyptus extracts (as Calyptol, Sanofi) has been the first product (and the most common product) used with WHV in our studies aimed to control the COVID virus. Calyptol produced a complete and fast negativization of swabs in a significant number of subjects (this will be presented in a separate paper in preparation) and it is considered the 'model', safe virucidal product to be used for viral respiratory diseases and for COVID patients in warm-humid vaporization).

This study is still in progress

Virucidals have been neglected so far, as they are cheap, not protected by patents and basically have an adjuvant role in a low-cost market. The real mission – almost impossible at the moment – is to selectively kill the virus in the body and outside, without side effects. Killing the virus early, in a preventive phase, could be the best option as advanced disease, with all its clinical complications, appear difficult to manage. We have to deal, probably, with this COVID problem for years, at least two years in prediction models [14-18]. The use of sequential antivirals (**Figure 2**), at low-dose in prevention may be more effective, safer and cheaper that the use of one simple antiviral in advanced patients (**Figure 2**). The concept is in evaluation and at the moment, results are not final.

Figure 2: killing the COVID virus early, in a preventive phase, could be the best option as advanced disease, with all its complications, appears very difficult to manage.



Possibly, we may have to deal with this problem for years, at least two years in prediction models. The cyclic use of sequential antivirals, at low-dose, in prevention, may be more effective and cheaper that the use of one sinmgleple antiviral in advanced patients and may cause minimal or no side effects. The concept is in evaluation. WHV allows vaporization with hot vapor, directly into the bronchial tree. Minor dosages (some 1/50-1/80 of the systemic dose) may be needed with minimal side effects and selectively targeting the most affected areas (bronchial tree).
Other possible (local) treatments may be added.

The combination of virucidal to block the viral spread and low-dose antivirals, if possible with other treatments may be the solution to control the pandemics. It is possible that the virus accumulates in salivary glands in some patients and are very difficult to eradicate The presence of viruses in the salivary glands may induce a longer period of contagiousness and may be associated to super spreading with individuals infecting for a long period of time a large number of individuals. In case of swelling, mild pain, in subjects otherwise considered asymptomatic, super spreading should be suspected and virucidals, used more often and more aggressively, may help in decrease contagiousness.

Mouth-dental risk of viral diffusion in asymptomatic patients is very high. The role of oral mucosa in COVID-19 infection and salivary glands in the epidemic process of asymptomatic infections is documented (19). ACE2, the important receptor for COVID-19, is very common in salivary gland epithelial cells; these cells have a high expression of ACE2 and can be easily infected. The expression of ACE2 in salivary glands is higher than in lungs; this suggests that salivary glands are a potential target for COVID-19 and act as an incubator. SARS-CoV RNA is detected in saliva (the first incubator) before lung lesions. This partially explains the presence of asymptomatic infections and spreaders. For SARS-CoV, the salivary glands are a major source of viruses in saliva. The positive rate of COVID-19 in patients' saliva can reach 91.7%, and saliva samples can also be used to cultivate the live virus. This indicates that COVID-19 may be transmitted by subjects with asymptomatic infections that originate, very often, from infected saliva. Most, persisting symptomatic infection (associated to super spreading) might be from salivary glands. Virucidals (Table 1) act where the virus is incubated (the incubators) and may have a very high community and management value to be studied in larger studies. In conclusion, virucidess may neutralize the virus in its primary incubators (mouth, saliva, salivary glands) and reduce the possibility of contamination.

Table 1: some virucides (some are shown in the list that may be very large) deactivate or destroy viruses. Most virucides are used on surfaces and may be used as cleaning agents to stop the spread of COVID-19. Some virucidals may be used to control the proliferation of viruses in humal surfaces and in 'viral incubators' (i.e., mouth).

- Isopropyl alcohol, ethanol, n-propanol
- Cyanovirin-N
- EP 0978289 A1 with iodine
- Griffithsin
- Interferon
- Lysol
- NVC-422
- Scytovirin
- Urumin
- Zonrox
- Vecoy Nanomedicines
- Liposomes with virucides
- Calyptol
- Australian tea tree oil
- Benzydamine
- Liquirice
- Pomagranate

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