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Inactivation of SARS-CoV-2 by povidone-iodine products: implications for effective mouth rinsing and gargling

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Abstract

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is transmitted mainly via droplets and contact. The implementation of infection control measures is important to reduce the number of COVID-19 cases. Thus, the ability of several povidone-iodine (PVP-I) products to inactivate SARS-CoV-2 was evaluated based on their *in vitro* inactivation efficacy. PVP-I solutions such as Isodine Gargle® (ethical and consumer products), Isodine Gargle C®, and Isodine Nodo Fresh® for 30 or 60 s decreased the viral infectivity level from 2-4 × 10^6 $TCID_{50}$/ml to below the detectable level (> 99.9% reduction). Our results indicate that the use of Isodine® mouthwash and gargle products is an effective infection control measure against SARS-CoV-2.

Key Words: COVID-19, povidoneiodine, SARS-CoV-2

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This disease emerged at the end of 2019[20,21] and expanded to all over the world as a pandemic within a short period. As of the end of April 2021, more than 150 million people have been infected, and nearly 3.2 million patients have died[10], resulting in the great impact to public health. COVID-19 has caused the largest pandemic since the "Spanish flu" in 1918–1919, which killed approximately 50 million people, with a mortality rate of more than 2.5%[19]. Several COVID-19 vaccines are in clinical trials, and some are now being administered to the public[7,15]. Antiviral agents are also used to treat patients[9]. The number of cases and deaths due to COVID-19, however, is still rising rapidly. Therefore, it is extremely important to prevent SARS-CoV-2 infection to reduce the number of patients and the burden on medical personnel in hospitals.

SARS-CoV-2 belongs to the genus *Betacoronavirus* within the family *Coronaviridae*, and its genome consists of positive-sense, single-stranded RNA surrounded by a lipid envelope[6]. Infection is initiated by binding between the

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Table 1. Table. Virucidal effect of Isodine products to SARS-CoV-2

Product name	Final PVP-I concentration (%)	Treatment time (sec)	Reduction in virus titer[1)]	
			(%)	(Log_{10})
Isodine Gargle (ethical product)	0.47	30	>99.94	>3.2
		60	>99.99	>4.0
Isodine Gargle (ethical product)	0.23	30	>99.93	>3.1
		60	>99.98	>3.6
Isodine Gargle (consumer product)	0.23	30	>99.92	>3.1
		60	>99.97	>3.6
Isodine Gargle C (consumer product)	0.35	30	>99.94	>3.2
		60	>99.96	>3.4
Isodine Nodo Fresh (consumer product)	0.45	30	>99.99	>3.8
		60	>99.99	>3.8

[1)] Reduction of $TCID_{50}$/ml to the control. $TCID_{50}$/ml of SARS-CoV-2 mixed with 0.5% sodium thiosulfate was regarded as the control value. The control values were around 2×10^6 to 4×10^6 $TCID_{50}$/ml.

viral spike (S) protein on the envelope and cellular angiotensin-converting enzyme 2[5)] and is promoted by cellular proteases such as TMPRSS2, TMPRSS11D, and TMPRSS13 after virus attachment to host cells[13,16)]. The virus may have originated from a bat species before it was transmitted to humans[17)], but it can be transmitted among humans, mainly via respiratory droplets and contact[14)]. Because SARS-CoV-2 is transmitted via respiratory droplets, the use of gargles and mouthwashes containing an effective disinfectant may reduce the viral load in the mouth, thus decreasing the risk of transmission. Various effective disinfectants against SARS-CoV-2 have been identified, including sodium hypochlorite, surfactants, and alcohols[11)]. However, these disinfectants are toxic and cannot be used on mucosal membranes.

Povidone-iodine (PVP-I) has been used as a disinfectant in gargles and mouthwashes as well as for wounds, and it was long ago proven safe for human use. PVP-I is effective against SARS-CoV[12)] and other RNA and DNA viruses[3,8,18)]. Therefore, it is likely that PVP-I may also inactivate SARS-CoV-2. In this study, we performed *in vitro* experiments to ascertain the inactivation efficacy of PVP-I products against SARS-CoV-2. These products are commercially available and widely used in medical and household settings. If PVP-I can effectively inactivate SARS-CoV-2, the use of gargle and mouthwash products containing PVP-I might be an efficient preventive measure for mitigating SARS-CoV-2 transmission.

The WK-521 strain of SARS-CoV-2 was kindly provided by Dr. Masayuki Saijo from the National Institute of Infectious Diseases. The virus was propagated in Vero E6 TMPRSS2 cells[16)] and cultured in Dulbecco's minimum essential medium (DMEM) containing 10% fetal bovine serum. Virus stocks were prepared by collecting culture supernatants from wells containing infected cells at 72 h post-infection, centrifuging the supernatants at 800 g for 10 min, and storing the clarified supernatants at –80 C until use.

Aliquots of virus stock solution (125 µl) with a 50% cell culture infectivity dose ($TCID_{50}$) of $1–6 \times 10^6$ $TCID_{50}$/ml were mixed with an equal volume of one of four PVP-I products. These included Isodine Gargle® (ethical and consumer products), Isodine Gargle C®, and Isodine Nodo Fresh® (Mundipharma, K. K.). Both Isodine Gargle products were diluted with distilled water to a recommended concentration for use before the addition of the virus. Isodine Gargle C and Isodine Nodo Fresh were added directly to equal volumes of the virus stock solution. The mixtures

were incubated for 30 or 60 s at room temperature and then diluted nine-fold with sodium thiosulfate (0.5%) to neutralize the cytotoxicity and antiviral activity of PVP-I. The mixtures were subsequently serially diluted in DMEM, and 0.1-ml aliquots were inoculated onto Vero E6 TMPRSS2 monolayers in 96-well plates. The cells were incubated for 72 h in a CO_2 incubator, after which the cells were observed under a microscope for signs of cytopathic effects. The $TCID_{50}$ of the virus–disinfectant mixture was determined following the method of Reed and Muench. All the experiments using infectious SARS-CoV-2 were carried out in a BSL3 containment room.

SARS-CoV-2 was exposed to various gargle products containing PVP-I to evaluate the virus inactivation efficacy of the products. All products, which comprised the ethical and consumer products of Isodine Gargle, Isodine Gargle C, and Isodine Nodo Fresh, reduced the abundance of the virus to below the detection level, which decreased the viral infectivity by more than 99.9% (Table). The results indicate that these PVP-I products exhibited strong virucidal efficacy against SARS-CoV-2. Hence, gargling with these products provides a protective measure against COVID-19.

COVID-19 emerged at the end of 2019 and rapidly spread worldwide. Although several vaccines have been developed, with some already being administered to the general public, the case and mortality rates are still very high. Therefore, it is necessary to implement effective preventive measures to protect against SARS-CoV-2 infection. PVP-I has been used as a disinfectant against various pathogens because of its high bactericidal and virucidal efficacies. Additionally, its use, especially in mouthwash and gargle products, does not cause substantial irritation to mucosal membranes[2]. PVP-I effectively inactivates SARS-CoV, which causes SARS in humans and has a close genetic relationship with SARS-CoV-2, being in the same genus *Betacoronavirus*[12]. Our results clearly demonstrated that PVP-I effectively inactivates SARS-CoV-2. The previous paper also reported the similar virucidal effect of PVP-I products to SARS-CoV-2 in vitro and suggested the usefulness of PVP-I in mouth hygiene[1]. The PVP-I products we tested are commercially available and commonly used in medical and household settings. SARS-CoV-2 is easily transmitted among humans via respiratory droplets and contact in enclosed places and close-contact settings. Clusters of COVID-19 cases are commonly reported to originate from nosocomial and household infections. Reducing the infection risk from hospital and household settings would greatly decrease the case rate. Our results imply that the use of mouthwashes and gargles containing PVP-I can markedly reduce the viral load excreted from patients. The use of mouthwashes or gargles containing PVP-I several times a day may protect against transmission between infected and non-infected individuals with high efficacy. A previous study also showed the *in vivo* protective efficacy of mouthwashes and gargles containing PVP-I in a clinical setting[4]. The use of mouthwashes containing PVP-I in patients should be evaluated in greater detail.

Conflict of interest statement

The authors declare no competing interests.

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