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Effects of Diluents, Saliva and Other Organics on the Microbicidal Activity of Cetylpyridinium Chloride and Povidone-iodine

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Povidone-iodine (PVP-I) is used for infection control and preoperative sterilization of the oral and pharyngeal regions. Marketed preparations containing cetylpyridinium chloride (CPC) are used to inhibit growth of oral bacteria. We conducted an *in vitro* study of the sterilizing effects of these microbicides on 10 oral bacterial strains and fungi related to pneumonia and periodontal disease, after dilution with phosphate-buffered saline (PBS), saliva, and components in saliva. The CPC solution was evaluated at 50 mg/100 mL, which is the concentration used in products. CPC sterilized all strains within 1 minute. Prolongation of the sterilization time associated with dilution was more gradual in comparison to PVP-I solution. CPC sterilized 7 of 10 microbial strains within 3 minutes at 3 mg/100 mL. At 500 mg/100 mL, which is near the upper limit of the concentration that is actually used, PVP-I solution sterilized 7 microbial strains within 3 minutes. However, PVP-I had no sterilization effect when diluted to 100 mg/100 mL or lower. With addition of saliva, PVP-I sterilized 2 microbial strains within 3 minutes at 500 mg/100 mL, whereas CPC solution sterilized 9 microbial strains within 1 minute at 50 mg/100 mL. Our results show that in use influenced by dilution with saliva, CPC is likely to maintain a strong sterilization effect, whereas PVP-I may have a reduced effect.

Key words : Cetylpyridinium chloride / Povidone-iodine / Aspiration pneumonia / Sterilization / Mouthwash.

INTRODUCTION

The incidence of aspiration pneumonia increases with advancing age, and the related factors include dysphagia, microbes in the oral cavity, and malnutrition (DiBardino and Wunderink, 2015). Oral care decreases the risk of aspiration pneumonia, and patients in health-care facilities who receive oral hygiene for 2 years have significantly lower mortality from pneumonia compared to patients who do not receive this care (Yoneyama et al., 1999). Furthermore, a multicenter, case-control study with propensity score matching analysis showed that perioperative oral care decreased the risk of post-operative pneumonia after esophageal cancer surgery

(Soutome et al., 2017). However, professional oral care requires dentists or dental hygienists. Self-care products are needed to allow patients to decrease oral microbes themselves and reduce the burden on medical staff at health-care facilities.

Povidone-iodine (PVP-I) is a microbicide that is commonly used in hospitals and homes to prevent oral and throat infection. However, gargling with PVP-I has been shown to be slightly less effective for throat infection compared to gargling with water, although with no significant difference (Satomura et al., 2005). A review of the effect of oral care with chlorhexidine (CHX) or PVP-I for prevention of ventilator-associated pneumonia showed that CHX was effective, whereas the effect of PVP-I was unclear (Labeau et al., 2011). PVP-I is readily inactivated by organics, including blood and saliva (Ogura et al., 1999), and its effect may be decreased

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by dilution with saliva in the oral cavity. However, despite increased discussion of the limitations of PVP-I, it is still used without clear knowledge of its effects.

Mouthwashes containing 0.12% or 0.2% CHX gluconate or hydrochloride are used as microbicides worldwide (Najafi et al., 2012). CHX is microbicidal for some microbes. For *S. mutans*, an MIC (minimum inhibitory concentration) of 70 mg/L (0.007%) and an MBC (minimum bactericidal concentration) of 150 mg/L (0.015%) have been reported (Uzer Celik et al., 2016). While the microbicidal effects of CHX on oral microorganisms have been frequently reported, anaphylactic shock at the time of use is a serious problem. In Japan, the 0.12% and 0.2% concentrations are not approved due to allergy concerns. The concentration of undiluted CHX-containing mouthwashes is restricted to 0.05% or less and they are used after dilution (Sugita et al., 2014). Mouthwashes, tooth pastes and oral agents for sore throat containing cetylpyridinium chloride (CPC), which is a cationic surfactant similar to CHX, are also commonly used. Anti-plaque and anti-gingivitis effects of 0.05% CPC products have been reported (Shim et al., 2012, Sreenivasan et al., 2013, Lee et al., 2017), and they are safely used worldwide. CPC is a long-acting microbicide that is absorbed on tooth surfaces (van der Mei et al., 1990) and is inactivated by organics (Takagi et al., 1989).

It is unclear how saliva and dilution influence the activities of CPC and PVP-I. Therefore, in this study, the microbicidal effects of PVP-I, CPC and commercial products containing these compounds and CHX were examined on oral microbial strains. Sterilization effects were compared after dilution with phosphate-buffered saline (PBS) and saliva, and after addition of components of saliva.

MATERIALS AND METHODS

Test preparations

PVP-I (Sigma-Aldrich, St. Louis, MO), CPC (Merck KGaA, Darmstadt, Germany), and the following commercial products were used in the study: PVP-I gargle containing 7% PVP-I to be diluted 15- to 30-fold (approximately 0.23% to 0.47%) at use; CPC mouthwash containing 0.05% CPC with glycyrrhizic acid dipotassium salt and benzalkonium chloride (CPC mouthwash A); CPC mouthwash containing 0.05% CPC with tranexamic acid (CPC mouthwash B); and CHX mouthwash containing 0.05% CHX to be diluted to 0.0001% to 0.0006% at use.

Microbial strains and culture method

The following 10 strains were used: pneumonia-related bacteria: *Streptococcus pneumoniae* ATCC33400

(*S.p.*), *Streptococcus intermedius* ATCC27335 (*S.i.*), *Streptococcus constellatus* subsp. *constellatus* ATCC27823 (*S.c.*), *Staphylococcus aureus* IFO12732 (*S.a.*), *Pseudomonas aeruginosa* IFO13275 (*P.a.*); periodontopathic bacteria: *Porphyromonas gingivalis* ATCC33277 (*P.g.*), *Fusobacterium nucleatum* ATCC25586 (*F.n.*), *Filifactor alocis* ATCC35896 (*F.a.*); dental caries pathogen: *Streptococcus mutans* ATCC25175 (*S.m.*); and fungi: *Candida albicans* NBRC1594 (*C.a.*). Culture conditions for microbes are shown in Table 1. The microbial strains were cultured on appropriate agar media and fluid media (10 mL).

Microbicidal tests of microbicides and commercial products

Microbicidal tests with inactivated solution containing 10% Tween80, 3% lecithin from soybean, and 0.5% sodium thiosulfate were performed to compare the microbicidal activities of PVP-I and CPC based on the time required for sterilization (Kunisada et al., 1999). Precultured microbes were suspended in appropriate media (10 mL) and adjusted to the McFarland No. 0.5 standard to prepare a microbial suspension. PVP-I or CPC were dissolved in distilled sterile water and diluted to eleven concentrations: 0.5, 1, 3, 5, 10, 30, 50, 100, 300, 500 and 1000 mg/100 mL. Commercial products were prepared as 2-, 3.75-, 7.5-, 15-, 30-, 60-, 120-, 240- and 480-fold diluted stock solutions.

Saliva, 1% (w/v) albumin, rabbit serum or PBS (100 µL) was added to diluted PVP-I, CPC or commercial products (100 µL). 1% (w/v) albumin or rabbit serum was added to some test microbes (*S.m.*, *S.a.*, *P.a.*, *S.p.*, *C.a.*). Each microbial suspension (20 µL) was added to the test solution and immediately mixed at room temperature. The mixture (20 µL) was collected 0.25, 0.5, 1, 3, 5, 10 and 30 min after mixing and inoculated into inactivated solution (180 µL). The resulting solution (20 µL) was inoculated into appropriate medium with 1% Tween80, 0.3% lecithin, 0.05% sodium thiosulfate (180 µL). After incubation for 72 hours under appropriate conditions for each microbe, growth was checked visually based on the turbidity of the broth. Additionally, *F. alocis* was inoculated on agar medium and viability was assessed by colony formation.

Saliva samples (40 mL) were collected from 3 healthy volunteers at rest from 9 to 11 am, mixed three times using a 23-G syringe, and centrifuged (10000 rpm, 4°C, 5 min). The supernatant (saliva) was sterilized with ultraviolet light for 1 h. Saliva samples were collected with attention to three ethical considerations: 1) collection was non-invasive, 2) was performed in healthy volunteers at Sunstar Inc., with their prior consent, and 3) saliva samples were mixed after being collected so that they could not be identified. Albumin

TABLE 1. Culture conditions for test microbes

microbe	Strain	Passage culture		Fluid culture		
		Agar medium	Medium	Culture condition	Time (hour)	
					Preculture	Culture after test
<i>P. gingivalis</i>	ATCC33277	Blood agar	TSB+Y/H	Anaerobic	48	72
<i>F. nucleatum</i>	ATCC25586		GAM		18	
<i>F. alocis</i>	ATCC35896		ATCC1490		72	
<i>S. mutans</i>	ATCC25175		BHI	Aerobic	18	
<i>S. pneumoniae</i>	ATCC33400					
<i>S. intermedius</i>	ATCC27335		TSB+Y/H	Anaerobic	24	
<i>S. constellatus</i>	ATCC27823					
<i>S. aureus</i>	IFO12732	BHI	BHI	Aerobic	18	
<i>P. aeruginosa</i>	IFO13275					
<i>C. albicans</i>	NBRC1594	Candida GS	Sabouraud			

BHI refers to Brain Heart Infusion, which is supplied as agar or broth. TSB+Y/H medium was supplemented with yeast extract (1 mg/mL), hemin (5 µg/mL), and vitamin K1 (0.5 µg/mL). GAM refers to Gifu Anaerobic Medium and was used as broth.

(Sigma-Aldrich, St. Louis, MO) was dissolved in distilled sterile water to prepare a 1% (w/v) solution. Stock solutions of rabbit serum (Biowest, Nuaille, France) were used in the study.

RESULTS

Sterilization times of CPC and PVP-I with test microbial strains

After adding PBS as a control, CPC and PVP-I solutions sterilized all microbial strains, including pneumonia-related bacteria, within 3 min at a concentration of 1000 mg/100 mL. Sterilization time increased with decreased concentrations of both agents. For diluted CPC, this increase was not rapid: 9 of 10 strains were sterilized within 30 s and all strains within 1 min at 30 mg/100 mL; and 7 of 10 strains within 3 min at 3 mg/100 mL. In contrast, PVP-I sterilized 7 of 10 strains within 3 min at 500 mg/100 mL, but the sterilizing effect decreased significantly at <100 mg/100 mL (Table 2-I).

Effects of added materials on sterilization times of CPC and PVP-I

Sterilization times of CPC and PVP-I solutions with added saliva were higher than those with added PBS. After addition of saliva, PVP-I sterilized 2 of 10 microbial strains at 500 mg/100 mL, whereas CPC sterilized 9 of 10 strains within 3 min at 30 mg/100 mL and did not show a rapid increase in sterilization time with dilution, compared with PVP-I (Table 2-II). After addition of 1% albumin, the CPC and PVP-I solutions sterilized all of 5 strains at 1000 mg/100 mL; however, CPC had a

sterilizing effect at a lower concentrations than PVP-I (Table 2-III). After addition of rabbit serum, CPC sterilized 2 of 5 strains at 500-1000 mg/100 mL, whereas PVP-I had no sterilizing effect on 5 strains (Table 2-IV).

Comparison of sterilization times of commercial products with test microbial strains

PVP-I gargle sterilized 7 of 10 microbial strains within 30 s at 467 mg/100 mL (15-fold dilution), the upper limit concentration for oral use. Sterilization effects decreased at 233 mg/100 mL (30-fold dilution), the lower limit concentration, at which PVP-I gargle had no effect on 8 of 10 strains. CPC mouthwashes A and B showed a slow increase in sterilization times at diluted concentrations (Table 3-I). CHX mouthwash sterilized all strains within 5 min at 50 mg/100 mL (stock solution), but had no sterilizing effect on all strains at 0.42 to 0.83 mg/100 mL (120 to 60 -fold dilution), which is close to the upper limit for oral use.

Effects on sterilization time of addition of saliva to commercial products

Saliva added to all commercial products prolonged the sterilization time (Table 3-II). All products were tested against the 10 microbial strains at concentrations for oral use. CPC mouthwashes A and B had sterilizing effects on 9 strains, PVP-I gargle sterilized 3 strains, and CHX mouthwash had no effect on any strains. At 2-fold dilution of the concentration for oral use, CPC mouthwashes A and B sterilized 8 strains within 5 min. PVP-I gargle sterilized 2 strains within 5 min, and CHX mouthwash had no sterilizing effect on any strains.

III. 1% albumin added

	mg/100 ml	0.5	1	3	5	10	30	50	100	300	500	1000
	%	0.0005	0.001	0.003	0.005	0.01	0.03	0.05	0.1	0.3	0.5	1
CPC	<i>S. mutans</i>	>30m					3m	15s				
	<i>S. aureus</i>	>30m						3m		1m		15s
	<i>P. aeruginosa</i>	>30m						30m	10m	1m		15s
	<i>S. pneumoniae</i>	>30m						30m	10m	1m		15s
	<i>C. albicans</i>	>30m					30m				5m	3m
	mg/100 ml	0.5	1	3	5	10	30	50	100	300	500	1000
	%	0.0005	0.001	0.003	0.005	0.01	0.03	0.05	0.1	0.3	0.5	1
PVP-I	<i>S. mutans</i>	>30m										30s
	<i>S. aureus</i>	>30m										30s
	<i>P. aeruginosa</i>	>30m										15s
	<i>S. pneumoniae</i>	>30m										15s
	<i>C. albicans</i>	>30m										15s

IV. Rabbit serum added

	mg/100 ml	0.5	1	3	5	10	30	50	100	300	500	1000
	%	0.0005	0.001	0.003	0.005	0.01	0.03	0.05	0.1	0.3	0.5	1
CPC	<i>S. mutans</i>	>30m							3m	15s		
	<i>S. aureus</i>	>30m								30m	30s	15s
	<i>P. aeruginosa</i>	>30m										
	<i>S. pneumoniae</i>	>30m										
	<i>C. albicans</i>	>30m								30m	10m	
	mg/100 ml	0.5	1	3	5	10	30	50	100	300	500	1000
	%	0.0005	0.001	0.003	0.005	0.01	0.03	0.05	0.1	0.3	0.5	1
PVP-I	<i>S. mutans</i>	>30m										
	<i>S. aureus</i>	>30m										
	<i>P. aeruginosa</i>	>30m										
	<i>S. pneumoniae</i>	>30m										
	<i>C. albicans</i>	>30m										

The shortest sterilization time for each microbicide is shown. s: second; m: minute

DISCUSSION

In this study, microbicidal effects were compared for CPC and PVP-I solutions diluted with PBS or saliva against oral microbial strains, including pneumonia-related bacteria and fungi. The shorter the time taken for sterilization, the higher the sterilizing effect of each product. After PBS was added, CPC sterilized all strains within 1 min at 50 mg/100 mL, which is the concentration actually used. CPC sterilized all strains within 1 min at 30 mg/100 mL and the sterilization time did not increase rapidly with dilution. PVP-I sterilized 4 to 7 strains within 3 min at concentrations of 300 to 500 mg/100 mL. These concentrations are close to the 15-fold dilution (467 mg/100 mL), which is the upper limit of the concentration actually used. However, PVP-I had no effect at 100 mg/100 mL or lower, which is lower than the lower limit (30-fold dilution, 233 mg/100 mL)

of the concentration actually used. In a study of several microbial strains using similar procedures (Katsukawa and Tamaru, 1996), *S. aureus* and *S. pneumoniae* were sterilized by CPC within 3 min at 5 and 1 mg/100 mL, respectively, and by PVP-I within 15 s and 3 min at 450 mg/100 mL. Our findings are consistent with the results of this previous study.

The sterilizing effect of PVP-I rapidly decreased after saliva was added, whereas that of CPC decreased less. After addition of saliva, PVP-I sterilized only 2 of 10 microbial strains at 500 mg/100 mL, whereas CPC sterilized 9 of 10 strains at 50 mg/100 mL (Table 2-II). To examine the effect of blood on microbicides, we added rabbit serum to the CPC and PVP-I solutions. This markedly decreased the sterilizing effects of both, but CPC still sterilized more microbial strains than PVP-I. In particular, CPC had stronger sterilizing effects on *S. mutans* and *S. aureus* compared to PVP-I; however,

TABLE 3. Sterilization times of commercial products with test microbe strains

I. Control

Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	<u>1</u>
	mg/100 ml	0.10	0.21	0.42	0.83	1.67	3.33	6.67	13.33	25	<u>50</u>
	%	0.0001	0.0002	0.0004	0.0008	0.0016	0.003	0.006	0.013	0.025	<u>0.05</u>
CPC mouthwash A	<i>P. gingivalis</i>	>30m			10m	3m		30s		15s	
	<i>F. nucleatum</i>	>30m				30m	3m		1m	30s	
	<i>F. alocis</i>	5m						3m	1m	30s	15s
	<i>S. mutans</i>	>30m			10m	5m	15s				
	<i>S. intermedius</i>	>30m		30m	10m	30s		15s			
	<i>S. constellatus</i>	>30m		10m	1m	30s		15s			
	<i>S. aureus</i>	>30m			30m	10m	5m				
	<i>P. aeruginosa</i>	>30m						30m		10m	
	<i>S. pneumoniae</i>	>30m		3m	30s	15s					
<i>C. albicans</i>		>30m									30m
Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	<u>1</u>
	mg/100 ml	0.10	0.21	0.42	0.83	1.67	3.33	6.67	13.33	25	<u>50</u>
	%	0.0001	0.0002	0.0004	0.0008	0.0016	0.003	0.006	0.013	0.025	<u>0.05</u>
CPC mouthwash B	<i>P. gingivalis</i>	>30m			30m	1m		30s		15s	
	<i>F. nucleatum</i>	>30m				10m		3m		1m	
	<i>F. alocis</i>	>30m		30m		10m	5m	3m		30s	15s
	<i>S. mutans</i>	>30m			30m	5m	15s				
	<i>S. intermedius</i>	>30m		5m		3m	1m	30s		15s	
	<i>S. constellatus</i>	>30m			5m	3m		30s	15s		
	<i>S. aureus</i>	>30m					5m	3m			
	<i>P. aeruginosa</i>	>30m						30m			
	<i>S. pneumoniae</i>	>30m			3m	1m	30s	15s			
<i>C. albicans</i>		>30m									30m
Dilution ratio (fold)		480	240	120	60	<u>30</u>	<u>15</u>	7.5	3.75	2	1
	mg/100 ml	14.58	29.17	58.33	117	<u>233</u>	<u>467</u>	933	1867	3500	7000
	%	0.01	0.03	0.05	0.11	<u>0.22</u>	<u>0.44</u>	0.875	1.75	3.5	7
PVP-I gargle	<i>P. gingivalis</i>	>30m					15s				
	<i>F. nucleatum</i>	>30m						15s			
	<i>F. alocis</i>	>30m						15s			
	<i>S. mutans</i>	>30m					15s				
	<i>S. intermedius</i>	>30m					30s	15s			
	<i>S. constellatus</i>	>30m					15s				
	<i>S. aureus</i>	>30m				30s	15s				
	<i>P. aeruginosa</i>	>30m					15s				
	<i>S. pneumoniae</i>	>30m			15s						
<i>C. albicans</i>		>30m					10m	15s			
Dilution ratio (fold)		<u>480</u>	<u>240</u>	<u>120</u>	60	30	15	7.5	3.75	2	1
	mg/100 ml	<u>0.10</u>	<u>0.21</u>	<u>0.42</u>	0.83	1.67	3.33	6.67	13.33	25	50
	%	<u>0.0001</u>	<u>0.0002</u>	<u>0.0004</u>	0.0008	0.0016	0.003	0.006	0.013	0.025	0.05
CHX mouthwash	<i>P. gingivalis</i>	>30m						3m	1m	15s	
	<i>F. nucleatum</i>	>30m					30m		30s		
	<i>F. alocis</i>	>30m					30m	5m	15s		
	<i>S. mutans</i>	>30m						3m		1m	15s
	<i>S. intermedius</i>	>30m						5m		30s	15s
	<i>S. constellatus</i>	>30m						3m		15s	
	<i>S. aureus</i>	>30m						10m		3m	30s
	<i>P. aeruginosa</i>	>30m					5m	30s	15s		
	<i>S. pneumoniae</i>	>30m					30m		1m	15s	
<i>C. albicans</i>		>30m						5m			

Dilution ratio (fold)		480	240	120	60	<u>30</u>	<u>15</u>	7.5	3.75	2	1	
	mg/100 ml	14.58	29.17	58.33	117	<u>233</u>	<u>467</u>	933	1867	3500	7000	
	%	0.01	0.03	0.05	0.11	<u>0.22</u>	<u>0.44</u>	0.875	1.75	3.5	7	
PVP-I gargle	<i>P. gingivalis</i>	>30m					15s					
	<i>F. nucleatum</i>	>30m							15s			
	<i>F. alocis</i>	>30m							15s			
	<i>S. mutans</i>	>30m					15s					
	<i>S. intermedius</i>	>30m					30s	15s				
	<i>S. constellatus</i>	>30m					15s					
	<i>S. aureus</i>	>30m				30s	15s					
	<i>P. aeruginosa</i>	>30m					15s					
	<i>S. pneumoniae</i>	>30m			15s							
	<i>C. albicans</i>	>30m					10m	15s				

Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	1
	mg/100 ml	<u>0.10</u>	<u>0.21</u>	<u>0.42</u>	0.83	1.67	3.33	6.67	13.33	25	50
	%	<u>0.0001</u>	<u>0.0002</u>	<u>0.0004</u>	0.0008	0.0016	0.003	0.006	0.013	0.025	0.05
CHX mouthwash	<i>P. gingivalis</i>	>30m						3m	1m	15s	
	<i>F. nucleatum</i>	>30m						30m	30s		
	<i>F. alocis</i>	>30m					30m	5m	15s		
	<i>S. mutans</i>	>30m							3m	1m	15s
	<i>S. intermedius</i>	>30m							5m	30s	15s
	<i>S. constellatus</i>	>30m							3m	15s	
	<i>S. aureus</i>	>30m							10m	3m	30s
	<i>P. aeruginosa</i>	>30m					5m	30s	15s		
	<i>S. pneumoniae</i>	>30m					30m	1m	15s		
	<i>C. albicans</i>	>30m								5m	

II. Saliva added

Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	<u>1</u>
CPC mouthwash A	mg/100 ml	0.10	0.21	0.42	0.83	1.67	3.33	6.67	13.33	25	50
	%	0.0001	0.0002	0.0004	0.0008	0.0016	0.003	0.006	0.013	0.025	0.05
	<i>P. gingivalis</i>	>30m					3m	1m	15s		
	<i>F. nucleatum</i>	>30m					5m	3m	30s		
	<i>F. alocis</i>	>30m			30m	5m		3m		30s	
	<i>S. mutans</i>	>30m				30m	3m	1m	30s	15s	
	<i>S. intermedius</i>	>30m			10m	5m			30s		15s
	<i>S. constellatus</i>	>30m				5m	3m	30s		15s	
	<i>S. aureus</i>	>30m				30m	10m	5m			
	<i>P. aeruginosa</i>	>30m								10m	1m
	<i>S. pneumoniae</i>	>30m			5m	1m		30s	15s		
	<i>C. albicans</i>	>30m						30m		10m	3m

Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	<u>1</u>
mg/100 ml		0.10	0.21	0.42	0.83	1.67	3.33	6.67	13.33	25	50
%		0.0001	0.0002	0.0004	0.0008	0.0016	0.003	0.006	0.013	0.025	0.05
CPC mouthwash B	<i>P. gingivalis</i>	>30m				10m	3m		15s		
	<i>F. nucleatum</i>	>30m					30m	10m	5m	3m	
	<i>F. alocis</i>	>30m					30m	3m		1m	15s
	<i>S. mutans</i>	>30m			30m	10m	1m	30s	15s		
	<i>S. intermedius</i>	>30m			10m	5m	1m		30s		
	<i>S. constellatus</i>	>30m			30m	3m		30s	15s		
	<i>S. aureus</i>	>30m					30m	10m	5m		
	<i>P. aeruginosa</i>	>30m							30m		
	<i>S. pneumoniae</i>	>30m			30m	5m	1m	30s	15s		
	<i>C. albicans</i>	>30m							30m	10m	3m

Dilution ratio (fold)		480	240	120	60	<u>30</u>	<u>15</u>	7.5	3.75	2	1
	mg/100 ml	14.58	29.17	58.33	117	233	467	933	1867	3500	7000
	%	0.01	0.03	0.05	0.11	0.22	0.44	0.875	1.75	3.5	7
PVP-I gargle	<i>P. gingivalis</i>	>30m				3m	30s				15s
	<i>F. nucleatum</i>	>30m						15s			
	<i>F. alocis</i>	>30m						30s	15s		
	<i>S. mutans</i>	>30m						15s			
	<i>S. intermedius</i>	>30m						30s	15s		
	<i>S. constellatus</i>	>30m						30s	15s		
	<i>S. aureus</i>	>30m					3m	1m	30s		
	<i>P. aeruginosa</i>	>30m				5m	3m	15s			
	<i>S. pneumoniae</i>	>30m				30m	5m	15s			
	<i>C. albicans</i>	>30m						10m	15s		

Dilution ratio (fold)		480	240	120	60	30	15	7.5	3.75	2	1
	mg/100 ml	<u>0.10</u>	<u>0.21</u>	<u>0.42</u>	0.83	1.67	3.33	6.67	13.33	25	50
	%	<u>0.0001</u>	<u>0.0002</u>	<u>0.0004</u>	0.0008	0.0016	0.003	0.006	0.013	0.025	0.05
CHX mouthwash	<i>P. gingivalis</i>	>30m						10m	1m	15s	
	<i>F. nucleatum</i>	>30m						3m	15s		
	<i>F. alocis</i>	>30m						1m	15s		
	<i>S. mutans</i>	>30m							3m	1m	30s
	<i>S. intermedius</i>	>30m						30m	5m	30s	15s
	<i>S. constellatus</i>	>30m							3m	15s	
	<i>S. aureus</i>	>30m							10m	3m	1m
	<i>P. aeruginosa</i>	>30m						10m	15s		
	<i>S. pneumoniae</i>	>30m						5m	15s		
	<i>C. albicans</i>	>30m					30m	10m	3m	15s	

The shortest sterilization time for each product is shown. The % indicates the concentration relative to the oral dose of the commercial product. s: second; m: minute

both CPC and PVP-I were less effective for other microbial strains. In this study, undiluted rabbit serum was added to evaluate the microbicidal effects of each microbicide when each was affected most strongly by serum. Sterilizing effects are likely to decrease in a poor periodontal environment with bleeding, and professional oral cleaning is required for patients with poor periodontal conditions. It is assumed that the oral cavity contains a mixture of saliva and blood, both in patients with periodontal diseases and in those with cancer of the mouth who are treated using radiotherapy. While the amount of saliva secreted and amount of bleeding derived from wounds and gingival inflammation in the oral cavity differ among individuals, the sterilizing effect of CPC is likely to be higher than that of PVP-I when the proportion of saliva increases, since the inhibitory effect of saliva was low on CPC, while PVP-I showed no sterilizing effect at 500 mg/100 mL when saliva was added. The effect of albumin, which is present in saliva and blood, on microbicides was also examined. Albumin is present at 4.2 to 5.1% in blood (Kanai et al., 2010), and 0.14 to 0.64% as the total protein in saliva (Shibuya et al., 1988). The efficacy of 1% albumin on various microbicides was investigated to include the total protein content (0.64%) in saliva. Addition of albumin increased the concentrations of CPC and PVP-I required to sterilize microbes. CPC sterilized 2 of 5 microbial strains at 50 mg/100 mL, whereas PVP-I had no microbicidal effect at 500 mg/100 mL.

The sterilizing effect of PVP-I occurs because free iodine has a strong oxidizing action; thus, a higher concentration of free iodine has a stronger sterilizing effect (Berkelman et al., 1982). Organic material may reduce free iodine, which decreases the oxidizing action and results in decreased reduction of microbes. In contrast, CPC is a microbicide with a cationic hydrophilic group that damages the surfaces of microbes, leading to sterilization (Ogura et al., 1999). The sterilizing effect may be decreased by formation of an ionic interaction between the positively charged CPC and organics; however, the results of this study suggest that organics including saliva and albumin had less effect on CPC than on PVP-I at concentrations for oral use. Clinical studies have shown unclear effects of PVP-I (Satomura et al., 2005; Labeau et al., 2011), and this may be because PVP-I at its oral dose does not have a sufficient sterilizing effect due to the effects of organics such as saliva in the oral cavity.

Similar tendencies to those for CPC and PVP-I were found for commercial products containing these compounds, with CPC products having stronger sterilizing effects than those containing PVP-I. In tests of other products, CHX mouthwash at the oral dose sterilized fewer microbial strains (Table 3-I). Mouthwashes

containing 0.12% or 0.2% CHX gluconate or hydrochloride are used as microbicides worldwide. The results of this study suggest pronounced sterilizing effects of CHX-containing products marketed overseas. All 10 microbial strains were sterilized within 5 minutes at a CHX concentration of 0.05%, which is the concentration in undiluted solution. A mouthwash product containing CHX used widely in dental clinics in Japan are diluted to 0.0001% to 0.0006% due to the concern of an allergic reaction to CHX. This dilution decreased the sterilizing effect in the conditions used in this study. The microbicidal effect of CHX was affected markedly by the working concentration, and CHX did not show a microbicidal effect when applied at low concentrations.

In conclusion, dilution or addition of saliva to PVP-I is likely to decrease the sterilization effect of an oral dose, whereas CPC and products containing CPC were influenced less by dilution and saliva, indicating that an oral dose of CPC has a sufficient sterilization effect. Commercial products containing CPC was influenced less by dilution and saliva compared to other products. The concentration CPC that is safely used in Japan has a sufficient sterilization effect even if saliva is present. We plan to conduct clinical studies to reveal the efficacies of CPC products.

REFERENCES

- Berkelman, R.L., Holland, B.W., and Anderson, R.L. (1982) Increased bactericidal activity of dilute preparations of povidone-iodine solutions. *J. Clin. Microbiol.*, **15**, 635-639.
- DiBardino, D.M., and Wunderink, R.G. (2015) Aspiration pneumonia: a review of modern trends. *J. Crit. Care.*, **30**, 40-48.
- Kanai, M., Okumura, N., Tozuka, M., and Yatomi, Y. (2010) *Clinical Laboratory Medicine, Thirty-Third Edition* (in Japanese). pp.406, Kanehara shuppan, Tokyo.
- Katsukawa, C., and Tamaru, A. (1996) A study of the antimicrobial effects of cetylpyridinium chloride (in Japanese). *Clinical Report*, **30**, 401-411.
- Kunisada, T., Yamada, K., Oda, S., and Hara, O. (1999) Study of a neutralizer system for in vitro evaluation of antiseptics (in Japanese). *Japanese Journal of Environmental Infections*, **14**, 127-131.
- Labeau, S.O., Van de Vyver, K., Brusselaers, N., Vogelaers, D., and Blot, S.I. (2011) Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. *Lancet Infect. Dis.*, **11**, 845-854.
- Lee, J.E., Lee, J.M., Lee, Y., Park, J.W., Suh, J.Y., Um, H.S., Kim, Y.G. (2017) The antiplaque and bleeding control effects of a cetylpyridinium chloride and tranexamic acid mouth rinse in patients with gingivitis. *J Periodontal Implant Sci.*, **47**, 134-142.
- Najafi, M.H., Taheri, M., Mokhtari, M.R., Forouzanfar, A., Farazi, F., Mirzaee, M., Ebrahimi, Z., and Mehrara, R. (2012) Comparative study of 0.2% and 0.12% digluconate chlorhexidine mouth rinses on the level of dental staining and gingival indices. *Dent. Res. J. (Isfahan)*, **9**, 305-308.
- Ogura, H., Kato, Y., and Shinoda, H. (1999) *Modern Dental Pharmacology* (in Japanese). pp.592, Ishiyaku shuppan,

- Tokyo.
- Satomura, K., Kitamura, T., Kawamura, T., Shimbo, T., Watanabe, M., Kamei, M., Takano, Y., and Tamakoshi, A. (2005) Prevention of upper respiratory tract infections by gargling: a randomized trial. *Am. J. Prev. Med.*, **29**, 302-307.
- Shibuya, M. (1988) Studies on Available Iodine (I₂) of PVP-I Solution and its Reaction with Saliva (in Japanese). *J Dent Hlth.*, **38**, 172-179.
- Shim, J.Y., Yim, S.B., Chung, J.H., and Hong, K.S. (2012) Antiplatelet and antigingivitis effects of a mouthrinse containing cetylpyridinium chloride, triclosan and dipotassium glycyrrhizinate. *J Periodontal Implant Sci.*, **42**, 33-38.
- Soutome, S., Yanamoto, S., Funahara, M., Hasegawa, T., Komori, T., Yamada, S.I., Kurita, H., Yamauchi, C., Shibuya, Y., Kojima, Y., Nakahara, H., Oho, T., and Umeda, M. (2017) Effect of perioperative oral care on prevention of postoperative pneumonia associated with esophageal cancer surgery: A multicenter case-control study with propensity score matching analysis. *Medicine (Baltimore)*, **96**, e7436.
- Sreenivasan, P.K., Haraszthy, V.I., and Zambon, J.J. (2013) Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. *Lett Appl Microbiol.*, **56**, 14-20.
- Sugita, N., Nakasone, N., Hanai, Y., Takahashi, M., Ito, H., Morozumi, T., Kubota, T., Okuda, K., and Yoshie, H. (2014) Microbiological effects of a low-concentration Chlorhexidine Hydrochloride/ cetylpyridinium chloride mouth rinse for patients with periodontitis (in Japanese). *Jpn J Conserv Dent.*, **57**, 219-228.
- Takagi, A., Saito, H., Kurimura, T., Kanemasa, Y., and Utsumi, S. (1989) *Essentials of Microbiology, Third Edition* (in Japanese). pp.132, Ishiyaku shuppan, Tokyo.
- Uzer Celik E., Tunac A.T., Ates M and Sen B.H. Antimicrobial activity of different disinfectants against cariogenic microorganisms. (2016) *Braz. Oral Res.*, **30**, e125.
- van der Mei, H.C., Perdok, J.F., Genet, M., Rouxhet, P.G., and Busscher, H.J. (1990) Cetylpyridinium chloride adsorption on the wettability and elemental surface composition of human enamel. *Clin. Prev. Dent.*, **12**, 25-29.
- Yoneyama, T., Yoshida, M., Matsui, T., and Sasaki, H. (1999) Oral care and pneumonia. Oral Care Working Group. *Lancet*, **354**, 515.