Original Article

JOMP
Journal of Oral Medicine and Pain

pISSN 2288-9272 eISSN 2383-8493
J Oral Med Pain 2018;43(1):1-7
https://doi.org/10.14476/jomp.2018.43.1.1

Influence of Hyaluronic Acid on the Different Levels of Lysozyme and Peroxidase in the Aspects of Candidacidal Activities

Jihoon Kim[1], Yoon-Young Kim[1], Ji-Youn Chang[1], Hong-Seop Kho[1,2]

[1]Department of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Korea
[2]Institute on Aging Seoul National University, Seoul, Korea

Received February 3, 2018
Revised March 5, 2018
Accepted March 7, 2018

Correspondence to:
Hong-Seop Kho
Department of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-3989
Fax: +82-2-744-9135
E-mail: hkho@snu.ac.kr

This study was supported by grant no. 04-2015-0080 from the Seoul National University Dental Hospital (SNUDH) Research Fund.

Purpose: The purpose of the study was to investigate the influences of hyaluronic acid on the candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase (GO-PO) system at different concentrations of antimicrobial enzymes.

Methods: Hyaluronic acid was used at a final concentration of 0.5 mg/mL. Hen egg-white lysozyme (HEWL) was used at concentrations ranging from 10 to 100 μg/mL. The peroxidase system included bovine lactoperoxidase (bLPO), potassium thiocyanate (KSCN, 1 mM), and hydrogen peroxide (100 μM). The GO-PO system included bLPO, KSCN (1 mM), glucose oxidase (10 units/mL), and glucose (30 μg/mL). The final concentration of bLPO in the peroxidase and GO-PO systems ranged from 12.5 to 100 μg/mL. *Candida albicans* strains ATCC 10231, 11006, and 18804 were utilized. Candidacidal activities of antimicrobials and the influence of hyaluronic acid on their candidacidal activities were determined based on colony forming units.

Results: Candidacidal activities of the peroxidase and GO-PO systems increased with increasing concentrations of bLPO. This tendency was the same in the presence or absence of hyaluronic acid. Candidacidal activity of HEWL was not significantly concentration-dependent. Candidacidal activities of the GO-PO system were higher than those of the corresponding peroxidase system. Candidacidal activity was inhibited in the presence of hyaluronic acid in the following order: HEWL, the peroxidase system, and the GO-PO system.

Conclusions: Hyaluronic acid inhibited the candidacidal activities of HEWL, the peroxidase system, and the GO-PO system. The GO-PO system exhibited better candidacidal activity than HEWL and the peroxidase system both in the presence and absence of hyaluronic acid.

Key Words: Candidacidal activity; Hyaluronic acid; Lysozyme; Peroxidase

INTRODUCTION

Many oral health care products contain synthetic and animal- or plant-derived products that aid in wound healing and boost antimicrobial activity.[1)] Hyaluronic acid, a glycosaminoglycan, is one such product used to boost wound healing and lubricating capacity[2)] and has been suggested as a candidate molecule for saliva substitutes for patients with dry mouth.[3)] Lysozyme and peroxidase of animal origin have also been used to replace or augment antimicrobial activity in these patients.[1)] Molecular interactions may occur between these components when they are included in one product.[4)] Such interactions are actually physiological phenomena, because hyaluronic acid, lysozyme, and peroxidase are all present in human saliva.[5,6)]

There have been several reports on the interactions between hyaluronic acid, lysozyme, and peroxidase from both structural and biological perspectives, and the reports on the biological aspect have focused on both enzymatic and antimicrobial activities.[3,4,7-9)] While structural interactions

Copyright © 2018 Korean Academy of Orofacial Pain and Oral Medicine. All rights reserved.

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

between hyaluronic acid and lysozyme[10-12] as well as between hyaluronic acid and peroxidase[13] have been suggested, hyaluronic acid itself does not alter the enzymatic activity of lysozyme and peroxidase.[3,13] However, hyaluronic acid has been reported to inhibit the enzymatic activity of the glucose oxidase-mediated peroxidase (GO-PO) system[9] which is commonly used in health care products instead of the peroxidase system.[1] This inhibitory effect of hyaluronic acid has been suggested to be due to inhibition of glucose oxidase activity.[9]

When hyaluronic acid, lysozyme, and peroxidase are incorporated in saliva substitutes to supplement the decreased oral antimicrobial activities in patients with dry mouth,[1] who have highly increased susceptibility for developing candidiasis, information concerning the interactions between hyaluronic acid, lysozyme, and peroxidase in terms of candidacidal activity is important. Hyaluronic acid has been reported to have fungistatic activity, but not fungicidal activity.[3,8,14] On the other hand, lysozyme, the peroxidase system, and the GO-PO system have fungicidal activities.[7,15-18] When these molecules are present in the same environment and interact, hyaluronic acid inhibits the candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system,[8,9] thereby limiting the advantages of blending hyaluronic acid with its viscoelastic properties into antimicrobial molecules with fungicidal activity. Thus, the purpose of the present study was to investigate the concentrations of antimicrobials needed to maintain candidacidal activity in mixtures containing hyaluronic acid.

MATERIALS AND METHODS

1. Hyaluronic Acid, Lysozyme, the Peroxidase System, and the Glucose Oxidase-Mediated Peroxidase System

Hyaluronic acid (1,630 kDa, Sigma-Aldrich, St. Louis, MO, USA) was used at a final concentration of 0.5 mg/mL in order to achieve similar viscoelastic properties as human saliva.[3] The concentrations of antimicrobials used in the experiments were determined based on previous studies that either investigated antimicrobial concentrations in human saliva or performed in vitro experiments to simulate biological events in the oral cavity.[7-9,16-20] For low concentration experiments, hen egg-white lysozyme (HEWL, Sigma-Aldrich) at a final concentration of 10 μg/mL and bovine lactoperoxidase (bLPO, Sigma-Aldrich) at a final concentration of 12.5 μg/mL were used as lysozyme and peroxidase sources, respectively. For high concentration experiments, HEWL and bLPO at final concentrations of both 50 μg/mL and 100 μg/mL were used. The peroxidase system included bLPO, potassium thiocyanate (KSCN, a final concentration of 1 mM), and hydrogen peroxide (H_2O_2, a final concentration of 100 μM). The GO-PO system included bLPO, KSCN (a final concentration of 1 mM), glucose oxidase (a final concentration of 10 units/mL, Sigma-Aldrich), and glucose (a final concentration of 30 μg/mL). All substances were solubilized with simulated salivary buffer (SSB, 0.021 M Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 36 mM NaCl and 0.96 mM $CaCl_2$). The results of candidacidal experiments at intermediate concentrations of 30 μg/mL HEWL and 25 μg/mL bLPO were from our previous studies.[8,9]

2. *Candida albicans* Strains

Three strains of *Candida albicans*, ATCC strains 10231, 11006, and 18804, were used to assess candidacidal assay at low (10 μg/mL of HEWL or 12.5 μg/mL of bLPO) and high concentrations (50 μg/mL of HEWL or bLPO) of antimicrobial enzymes. For very high concentration experiments (100 μg/mL of HEWL or bLPO), only *C. albicans* strain 18804 was used.

3. Influence of Hyaluronic Acid on the Candidacidal Activity of Lysozyme, the Peroxidase System, and the GO-PO System

The influence of hyaluronic acid on the candidacidal activity of antimicrobials was investigated using two different sequences: (1) pre-incubation of hyaluronic acid with candidal cells followed by incubation with antimicrobials, and (2) pre-incubation of hyaluronic acid with antimicrobials followed by incubation with candidal cells. The experiments with lysozyme and the peroxidase system were performed eight times and those with the GO-PO system were performed six times.

1) Pre-incubated mixture of hyaluronic acid and candidal cells was added to antimicrobials

One colony of *C. albicans* grown on Sabouraud dextrose

agar (SDA) was inoculated into 10 mL Sabouraud dextrose broth and incubated with shaking at 37°C for 18 h. Cells were then harvested, washed, and resuspended to a concentration of 1×10^5 cells per mL in SSB. Next, 20 μL of the cell suspension was added to an equal volume of hyaluronic acid. The samples were then incubated with shaking at 37°C for 1 h, after which 40 μL of the cell suspension was mixed with 20 μL of HEWL followed by incubation with shaking at 37°C for another 1 h. The same volume of the peroxidase system or the GO-PO system instead of HEWL was used to investigate the influence of hyaluronic acid on the antimicrobial systems. After incubation, the mixtures were diluted 10-fold, and 50 μL (167 cells) of the diluted cells were plated onto SDA plates in triplicate and grown overnight at 37°C. The influence on candidacidal activity was determined by comparing the number of colonies (colony forming units, CFUs) on experimental (with antimicrobials and hyaluronic acid) and control plates (with antimicrobials and without hyaluronic acid). The percent loss of cell viability was calculated as 1 minus the ratio of the number of colonies on the experimental or control plates to that on the blank plates (without antimicrobials and hyaluronic acid).

2) Pre-incubated mixture of hyaluronic acid and antimicrobials was added to candidal cells

We added 20 μL of hyaluronic acid solution to 20 μL of HEWL (or the peroxidase system, or the GO-PO system) and incubated the mixture with shaking at 37°C for 1 h. The mixture was then added to 20 μL of the cell suspension and incubated with shaking at 37°C for another 1 h. After incubation, the sample was diluted 10-fold, and 50 μL of the diluted cells were plated onto SDA plates in triplicate and grown overnight at 37°C.

4. Statistics

The Wilcoxon signed rank test and Mann-Whitney U test were used to analyze statistical differences between variables. The Kruskal-Wallis test was used to analyze statistical differences, and the Mann-Whitney U test was used for post-hoc analysis. p-values less than 0.05 were considered statistically significant.

RESULTS

1. Influence of Hyaluronic Acid on the Candidacidal Activities of Lysozyme at Different Lysozyme Concentrations

HEWL exhibited 20%-30% candidacidal activity at the concentration of 10-50 μg/mL, and there were no significant differences in activity according to the concentration of HEWL and pre-incubation sequence (Tables 1, 2). The candidacidal activities of HEWL at 10 μg/mL were almost completely inhibited by the presence of hyaluronic acid, while those at 50 μg/mL exhibited the levels of 5.9%-18.7%, with relatively greater inhibitory activity towards the 10231 strain (Tables 1, 2). There were no significant differences in the candidacidal activities of HEWL between 50 and 100 μg/mL, nor were any significant differences in the inhibitory effects of hyaluronic acid at these concentrations (Supplementary Table 1; available online only).

Table 1. Influence of HA on the candidacidal activity of lysozyme according to the concentration of HEWL. HA was pre-incubated with candidal cells, followed by treatment with HEWL

Candida albicans strain	N=8	10 μg/mL HEWL		30 μg/mL HEWL*		50 μg/mL HEWL		Significance among groups	
		HEWL	With HA	HEWL	With HA	HEWL	With HA	HEWL	With HA
ATCC 10231	% killing	19.2±7.6	0.1±7.4	28.7±17.3	15.9±22.3	20.4±6.9	5.9±7.9	NS	NS
ATCC 11006	% killing	19.9±10.6	1.3±7.0[a]	32.6±16.2	4.6±9.5[b]	25.9±6.4	14.0±8.5[a,b]	NS	p<0.05
ATCC 18804	% killing	18.1±9.8	1.2±4.1[c]	26.9±6.7	3.3±16.2	22.6±10.0	15.4±6.1[c]	NS	p<0.05

HA, hyaluronic acid; HEWL, hen egg-white lysozyme; NS, not significant.
Values are presented as mean±standard deviation.
The Kruskal-Wallis test was used to analyze differences according to the concentration of HEWL. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 30 μg/mL of HEWL were from our previous study.[8]

Table 2. Influence of HA on the candidacidal activity of lysozyme according to the concentration of HEWL. HA was pre-incubated with HEWL, followed by treatment with candidal cells

Candida albicans strain	N=8	10 μg/mL HEWL		30 μg/mL HEWL*		50 μg/mL HEWL		Significance among groups	
		HEWL	With HA	HEWL	With HA	HEWL	With HA	HEWL	With HA
ATCC 10231	% killing	25.2±9.4	2.5±12.6	26.6±8.9	5.3±14.2	19.5±9.9	11.5±9.8	NS	NS
ATCC 11006	% killing	19.7±7.5	0.0±10.0[a]	21.5±8.9	1.5±11.3[b]	19.6±5.2	16.9±6.1[a,b]	NS	p<0.05
ATCC 18804	% killing	20.8±10.4	0.8±4.7[c]	26.9±11.8	3.4±14.6[d]	22.2±7.5	18.7±6.6[c,d]	NS	p<0.05

HA, hyaluronic acid; HEWL, hen egg-white lysozyme; NS, not significant.
Values are presented as mean±standard deviation.
The Kruskal-Wallis test was used to analyze differences according to the concentration of HEWL. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 30 μg/mL of HEWL were from our previous study.[8)]

Table 3. Influence of HA on the candidacidal activity of the peroxidase system according to the concentration of bLPO. HA was pre-incubated with candidal cells, followed by treatment with the peroxidase system

Candida albicans strain	N=8	12.5 μg/mL bLPO		25 μg/mL bLPO*		50 μg/mL bLPO		Significance among groups	
		Peroxidase system	With HA	Peroxidase system	With HA	Peroxidase system	With HA	Peroxidase system	With HA
ATCC 10231	% killing	24.0±16.3	4.3±12.1	15.9±8.4	6.0±13.9	22.0±12.4	11.4±8.8	NS	NS
ATCC 11006	% killing	20.0±11.6[a]	1.7±13.4	37.4±7.7[a]	5.8±6.4	20.3±4.6	11.5±8.3	p<0.05	NS
ATCC 18804	% killing	15.2±6.9[b]	2.7±12.1	25.7±7.3[b]	14.1±12.5	17.9±5.6	10.0±8.9	p<0.05	NS

HA, hyaluronic acid; bLPO, bovine lactoperoxidase; NS, not significant.
Values are presented as mean±standard deviation.
The peroxidase system was completed by addition of potassium thiocyanate (KSCN, at a final concentration of 1 mM), and hydrogen peroxide (H_2O_2, at a final concentration of 100 μM).
The Kruskal-Wallis test was used to analyze differences according to the concentration of bLPO. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 25 μg/mL of bLPO were from our previous study.[8)]

Table 4. Influence of HA on the candidacidal activity of the peroxidase system according to the concentration of bLPO. HA was pre-incubated with the peroxidase system, followed by treatment with candidal cells

Candida albicans strain	N=8	12.5 μg/mL bLPO		25 μg/mL bLPO*		50 μg/mL bLPO		Significance among groups	
		Peroxidase system	With HA	Peroxidase system	With HA	Peroxidase system	With HA	Peroxidase system	With HA
ATCC 10231	% killing	13.4±12.8	7.1±10.7	23.2±13.3	13.0±17.6	17.5±6.1	9.1±5.1	NS	NS
ATCC 11006	% killing	28.5±7.6	5.1±7.1[a]	34.0±11.9	14.1±12.1	21.0±8.5	18.2±8.2[a]	NS	p<0.05
ATCC 18804	% killing	19.1±11.0[b]	1.1±6.7[c,d]	29.2±5.0[b]	12.1±9.8[c]	24.1±5.0	14.4±8.4[d]	p<0.05	p<0.05

HA, hyaluronic acid; bLPO, bovine lactoperoxidase; NS, not significant.
Values are presented as mean±standard deviation.
The peroxidase system was completed by addition of potassium thiocyanate (KSCN, at a final concentration of 1 mM), and hydrogen peroxide (H_2O_2, at a final concentration of 100 μM).
The Kruskal-Wallis test was used to analyze differences according to the concentration of bLPO. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 25 μg/mL of bLPO were from our previous study.[8)]

2. Influence of Hyaluronic Acid on the Candidacidal Activities of the Peroxidase System at Different Peroxidase Concentrations

There was a small increase in the candidacidal activity of the peroxidase system as the concentration of bLPO increased, and strain-specific differences were noted. In the presence of hyaluronic acid, the candidacidal activities of the peroxidase system at 25 or 50 μg/mL of bLPO were higher compared to those at 12.5 μg/mL of bLPO irrespective of pre-incubation sequence, although there was no

significant difference between the concentrations of 25 and 50 μg/mL (Tables 3, 4). There were no significant differences in the candidacidal activities of the peroxidase system and the inhibitory effects of hyaluronic acid on the system between 50 and 100 μg/mL bLPO (Supplementary Table 1).

3. Influence of Hyaluronic Acid on the Candidacidal Activity of the GO-PO System at Different Peroxidase Concentrations

The GO-PO system with and without hyaluronic acid exhibited higher candidacidal activity than did the corresponding peroxidase system. The candidacidal activities of the GO-PO system at a concentration of 12.5-50 μg/mL bLPO were approximately 30%-40%, while in the presence of hyaluronic acid the activity was about 20%-30% (Tables 5, 6). The candidacidal activity of the GO-PO system at 50 μg/mL of bLPO was increased compared to those at 12.5 or 25 μg/mL of bLPO, and strain-specific differences were noted (Tables 5, 6). There were no significant differences in the candidacidal activity of the GO-PO system and the inhibitory effects of hyaluronic acid for bLPO concentrations of 50 and 100 μg/mL (Supplementary Table 1).

DISCUSSION

Hyaluronic acid, lysozyme, and the peroxidase system are present in human saliva, and therefore interactions among these molecules are physiological phenomena. However, the concentration of hyaluronic acid in unstimulated whole saliva is very low, approximately 400 ng/mL,[5] and its effects on other molecules in saliva are thought to be minimal. Hyaluronic acid, lysozyme, and the GO-PO system are used

Table 5. Influence of HA on the candidacidal activity of the GO-PO system according to the concentration of bLPO. HA was pre-incubated with candidal cells, followed by treatment with the GO-PO system

Candida albicans strain	N=6	12.5 μg/mL bLPO		25 μg/mL bLPO*		50 μg/mL bLPO		Significance among groups	
		GO-PO system	With HA	GO-PO system	With HA	GO-PO system	With HA	GO-PO system	With HA
ATCC 10231	% killing	30.8±5.0[a]	19.9±3.4	27.8±3.7[b]	16.4±11.7	41.5±8.4[a,b]	25.3±8.3	p<0.05	NS
ATCC 11006	% killing	30.1±5.4	19.7±4.7[c]	35.0±5.9	20.1±8.6[d]	41.4±10.0	34.9±9.1[c,d]	NS	p<0.05
ATCC 18804	% killing	32.6±9.3	23.6±5.5	30.1±6.9	18.1±14.7	42.6±10.8	28.3±12.7	NS	NS

HA, hyaluronic acid; GO-PO, glucose oxidase-mediated peroxidase; bLPO, bovine lactoperoxidase; NS, not significant.
Values are presented as mean±standard deviation.
The GO-PO system was completed by addition of potassium thiocyanate (KSCN, at a final concentration of 1 mM), glucose oxidase (at a final concentration of 10 units/mL), and glucose (at a final concentration of 30 μg/mL).
The Kruskal-Wallis test was used to analyze differences according to the concentration of bLPO. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 25 μg/mL of bLPO were from our previous study.[9]

Table 6. Influence of HA on the candidacidal activity of the GO-PO system according to the concentration of bLPO. HA was pre-incubated with the GO-PO system, followed by treatment with candidal cells

Candida albicans strain	N=6	12.5 μg/mL bLPO		25 μg/mL bLPO*		50 μg/mL bLPO		Significance among groups	
		GO-PO system	With HA	GO-PO system	With HA	GO-PO system	With HA	GO-PO system	With HA
ATCC 10231	% killing	31.5±13.0	22.1±7.8	34.9±14.6	27.0±9.2	29.5±9.0	23.9±8.4	NS	NS
ATCC 11006	% killing	36.7±3.0	21.6±9.8	37.8±5.6	22.8±5.7	33.8±4.9	30.0±6.2	NS	NS
ATCC 18804	% killing	37.5±8.2	23.7±10.4	28.8±9.2	15.8±11.7[a]	36.6±4.5	31.6±4.3[a]	NS	p<0.05

HA, hyaluronic acid; GO-PO, glucose oxidase-mediated peroxidase; bLPO, bovine lactoperoxidase; NS, not significant.
Values are presented as mean±standard deviation.
The GO-PO system was completed by addition of potassium thiocyanate (KSCN, at a final concentration of 1 mM), glucose oxidase (at a final concentration of 10 units/mL), and glucose (at a final concentration of 30 μg/mL).
The Kruskal-Wallis test was used to analyze differences according to the concentration of bLPO. The Mann-Whitney U test was used for post hoc analysis. Same letter pairs denote a significant difference. p<0.05.
*The results for 25 μg/mL of bLPO were from our previous study.[9]

in oral health care products, in which the concentration of hyaluronic acid is 1-2 mg/mL. Therefore, interactions among these molecules need to be investigated in various biological and rheological aspects. The purpose of this study was to investigate the concentrations of HEWL and bLPO needed to maintain candidacidal activity in the presence of 0.5 mg/mL hyaluronic acid, which mimics the viscoelastic properties of human saliva.[3] Interestingly, the candidacidal activities of HEWL, the peroxidase system, and the GO-PO system in the low concentration experiments were not very different from the activities observed for intermediate and high concentration experiments.

Several mechanisms have been proposed to explain the candidacidal effects of lysozyme and peroxidase, including direct bindings, interaction with yeast cell-wall components, and de-regulation of fungal enzymes or influx-efflux systems.[15,16,21-23] These mechanisms are not likely to be specific, and the candidacidal activities of these antimicrobial systems appeared to approach a plateau in the low concentration experiments. However, in the presence of 0.5 mg/mL hyaluronic acid, the candidacidal activities of HEWL at 10 μg/mL and of the peroxidase system at 12.5 μg/mL bLPO were almost completely inhibited. A minimum of 30-50 μg/mL of HEWL and 25-50 μg/mL of bLPO was needed to achieve appreciable candidacidal activity in the presence of hyaluronic acid.

On the other hand, the candidacidal activities of the GO-PO system were higher than those of the peroxidase system at the same bLPO concentrations both in the presence and absence of hyaluronic acid. Specifically, when hyaluronic acid was present, the GO-PO system exhibited a certain level of candidacidal activity even at 12.5 μg/mL bLPO. Because H_2O_2 can be produced by utilizing glucose, the GO-PO system is used as an actual component of health care products rather than the peroxidase system. In this study, we used glucose at a concentration of 30 μg/mL, which is the physiological concentration of glucose in human saliva.[24,25] Therefore, our results suggest that the GO-PO system with 0.5 mg/mL of hyaluronic acid could exhibit candidacidal activity in the oral cavity by utilizing salivary glucose. Although verification of this possibility requires in vivo studies, the GO-PO system has an advantage as a component of oral health care products, especially those containing hyaluronic acid.

Considering that our results did not differ significantly according to the incubation sequence, the inhibitory mechanism of hyaluronic acid appeared to be inhibition of diffusion of antimicrobials by its large molecular size.[8] Therefore, the candidacidal activities of the antimicrobial systems in the presence of hyaluronic acid were increased as the levels of antimicrobials were increased, although they eventually reached a plateau. For example, the candidacidal activities of the antimicrobial systems in the presence of hyaluronic acid at 50 μg/mL of either HEWL or bLPO were almost the same as those at the 100 μg/mL. This information may be important for determining the concentrations of antimicrobial supplements in health care products containing hyaluronic acid.

The results of this study were based on one kind of antimicrobial or antimicrobial system utilizing different concentration of HEWL or bLPO. Thus, it will be necessary to obtain further information about the candidacidal activities at different concentrations of other components in the peroxidase system and the GO-PO system. Considering the possibility of synergism between different antimicrobials, further information is also needed regarding the candidacidal activities in the case of combined use of antimicrobials.

In conclusion, hyaluronic acid inhibited the candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system. The GO-PO system showed better candidacidal activity than the peroxidase system both in the presence and absence of hyaluronic acid. The GO-PO system can be considered a proper antimicrobial component of oral health care products, especially those containing hyaluronic acid.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Tenovuo J. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: efficacy and safety. Oral Dis 2002;8:23-29.
2. Almond A. Hyaluronan. Cell Mol Life Sci 2007;64:1591-1596.
3. Park MS, Chang JY, Kang JH, Park KP, Kho HS. Rheological prop-

erties of hyaluronic acid and its effects on salivary enzymes and candida. Oral Dis 2010;16:382-387.
4. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000;14:40-47.
5. Pogrel MA, Lowe MA, Stern R. Hyaluronan (hyaluronic acid) in human saliva. Arch Oral Biol 1996;41:667-671.
6. Pogrel MA, Low MA, Stern R. Hyaluronan (hyaluronic acid) and its regulation in human saliva by hyaluronidase and its inhibitors. J Oral Sci 2003;45:85-91.
7. Lee JY, Kim YY, Chang JY, Park MS, Kho HS. The effects of peroxidase on the enzymatic and candidacidal activities of lysozyme. Arch Oral Biol 2010;55:607-612.
8. Kang JH, Kim YY, Chang JY, Kho HS. Influences of hyaluronic acid on the anticandidal activities of lysozyme and the peroxidase system. Oral Dis 2011;17:577-583.
9. Cho MA, Kim YY, Chang JY, Kho HS. Interactions between hyaluronic acid, lysozyme, and the glucose oxidase-mediated lactoperoxidase system in enzymatic and candidacidal activities. Arch Oral Biol 2013;58:1349-1356.
10. Van Damme MP, Moss JM, Murphy WH, Preston BN. Binding of hyaluronan to lysozyme at various pHs and salt concentrations. Biochem Int 1991;24:605-613.
11. Van Damme MP, Moss JM, Murphy WH, Preston BN. Binding properties of glycosaminoglycans to lysozyme--effect of salt and molecular weight. Arch Biochem Biophys 1994;310:16-24.
12. Moss JM, Van Damme MP, Murphy WH, Preston BN. Dependence of salt concentration on glycosaminoglycan-lysozyme interactions in cartilage. Arch Biochem Biophys 1997;348:49-55.
13. Green SP, Baker MS, Lowther DA. Depolymerization of synovial fluid hyaluronic acid (HA) by the complete myeloperoxidase (MPO) system may involve the formation of a HA-MPO ionic complex. J Rheumatol 1990;17:1670-1675.
14. Sakai A, Akifusa S, Itano N, et al. Potential role of high molecular weight hyaluronan in the anti-*Candida* activity of human oral epithelial cells. Med Mycol 2007;45:73-79.
15. Marquis G, Montplaisir S, Garzon S, Strykowski H, Auger P. Fungitoxicity of muramidase. Ultrastructural damage to *Candida albicans*. Lab Invest 1982;46:627-636.
16. Tobgi RS, Samaranayake LP, MacFarlane TW. In vitro susceptibility of Candida species to lysozyme. Oral Microbiol Immunol 1988;3:35-39.
17. Lenander-Lumikari M. Inhibition of Candida albicans by the Peroxidase/SCN-/H_2O_2 system. Oral Microbiol Immunol 1992;7:315-320.
18. Kho HS, Kim YY, Chang JY, Kim MJ, Lee SG. Candidacidal activities of the glucose oxidase-mediated lactoperoxidase system. Arch Oral Biol 2012;57:684-688.
19. Tenovuo JO. Nonimmunoglobulin defense factors in human saliva. In: Tenovuo JO, ed. Human saliva: clinical chemistry and microbiology. Boca Raton: CRC Press; 1989. pp. 55-91.
20. Korpela A, Yu X, Loimaranta V, et al. Lactoperoxidase inhibits glucosyltransferases from *Streptococcus mutans* in vitro. Caries Res 2002;36:116-121.
21. Petit JF, Jolles P. Purification and analysis of human saliva lysozyme. Nature 1963;200:168-169.
22. Marquis G, Garzon S, Strykowski H, Auger P. Cell walls of normal and lysozyme-damaged blastoconidia of *Candida albicans*: localization of surface factor 4 antigen and vicinal-glycol staining. Infect Immun 1991;59:1312-1318.
23. Wright CD, Bowie JU, Gray GR, Nelson RD. Candidacidal activity of myeloperoxidase: mechanisms of inhibitory influence of soluble cell wall mannan. Infect Immun 1983;42:76-80.
24. Jurysta C, Bulur N, Oguzhan B, et al. Salivary glucose concentration and excretion in normal and diabetic subjects. J Biomed Biotechnol 2009;2009:430426.
25. Soares MS, Batista-Filho MM, Pimentel MJ, Passos IA, Chimenos-Küstner E. Determination of salivary glucose in healthy adults. Med Oral Patol Oral Cir Bucal 2009;14:e510-e513.