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博士學位論文

The Anti-sticking Effect of Mixture of Trisodium Phosphate and Citric Acid on Oral Streptococcus species.

朝鮮大學校 大學院

醫學科

趙 炯 勳

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*2008*年 *8*月 *25*日

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朝鮮大學校 大學院

국문 초록

구연산과 제3 인산나트륨 혼합액의 구강 내 사슬알균에 대한 항 부착 능력

The Anti-sticking Effect of Mixture of Trisodium Phosphate and Citric Acid on Oral Streptococcus species.

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제3 인산나트륨(12H_2O, SIGMA, USA)과 구연산 (1H_2O, SIGMA, USA)의 혼합물은 직경 7㎜의 유리구슬에 부착된 *Streptococcus mutans* (KCTC 3065), *Streptococcus mitis* (KCTC 3556)와 *Streptococcus salivarius* (KCTC 3960)에 대하여 강력한 항 부착 효과를 나타내었다. 상기 사슬알균들은 3개의 유리구슬들이 함유된 brain heart infusion broth 배지에서 18시간 동안 흔들 배양된 후, 3개 핀점이 부착된 핀셋으로 집어서 생리식염수에서 가볍게 세척되었다. 이어서 각각의 유리구슬들을 4가지 시약들이 함유된 시험관들에 옮겼다. 각 시험관들에 사람이 칫솔질을 하는 것과 유사한 효과를 유발하기 위해, 물에 젖어도 잘 풀어지지 않는 종이 조각(2-3mm 크기) 40mg을 넣었다. 시험관들을 vortex mixer로 레벨 10에서 10분간 흔들었다. 예외로 비구강 사슬알균인 *Streptococcus agalactiae*는 5분간 흔들었다. 각 시험관들에서 용액 샘플을 소량 취하여 한천 계단 희석법을 사용하여 배양하였다. 각 사슬알균 종에 대하여 3회 반복 실험하였으며, 시약에 의하여 탈부착되는 비율은 생리식염수를 사용한 대조군에서 탈부착된 균의 량과 비교하였다. *Streptococcus mutans*에 대한 구연산-제3인산나트륨-생리식염수 혼합액(CTS, pH 6.0)의 탈부착 효과는 생리식염수 대조군에 비하여 평균 12.5배였다. 제3인산나트륨-생

리식염수 혼합액(TS, pH 8.4)의 탈부착 효과는 생리식염수 대조군에 비하여 7.5배였고, 구연산-생리식염수 혼합액(CS, pH 4.6)은 6.0배였다. *Streptococcus salivarius*에 대해서는 상기 시약의 순서대로 각 7.2배, 2.6배, 2.8배였으며, *Streptococcus mitis*에 대해서는 각 2.4배, 3.4배, 0.3배였다. 비구강 사슬알균인 *Streptococcus agalactiae*에 대해서는 각 0.7, 0.6, 0.6으로 생리식염수 대조군에 비하여 오히려 탈부착 효과가 크게 감소하였다. 이러한 결과는 충치와 아급성 심내막염의 주요 원인균인 구강내 *Streptococcus mutans*와 *Streptococcus salivarius*가 구연산-제3인산나트륨-생리식염수 혼합액에 의하여 구강 생태계로부터 쉽게 제거될 수 있다는 사실을 간접적으로 시사해 주고 있다. 따라서 본 실험 결과를 응용하면 발치 후에 발병율이 높은 아급성 심내막염과 충치의 예방에 이용할 수 있는 새로운 개념의 치약을 개발할 수 있을 것으로 추론할 수 있다.

Keywords: citric acid, trisodium phosphate, dental caries, *Streptococcus spp.*

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INTRODUCTION

Trisodium phosphate (TSP, Na_3PO_4) is chemically classified as GRAS (generally recognized as safe) by Food and Drug Administration(FDA) of United States and is world-widely used in food hygiene. A process using a food grade ortho-phosphate (trisodium phosphate, Na_3PO_4) to reduce viable *Salmonella* in chicken has been approved by the US Department of Agriculture under the conditions proposed for treatment (immersion of processed poultry in 8-12% solutions of TSP for 15 seconds). Many researchers have examined the decontamination of pathogenic bacteria like *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella spp*. etc. in several poultry, beef, and eggs. They have examined acetic acid or lactic acid with TSP solution and reported good decontamination results. But the concentrations of acid and TSP that were used on food are too high to apply to oral microbes research. According to literature survey, there are no reports that examined low concentration of citric acid and TSP for the study of oral microbes. Author of this paper devised the experimental model that can measure the degree of de-adherence against three oral *Streptococcus spp*. and one non-oral *Streptococcus species*, and examined the effect of TSP or citric acid solution. The aim of this study is to evaluate whether the low concentrated TSP or citric acid solution influence oral *Streptococcus* species that cause dental caries and subacute endocarditis.

MATERIALS AND METHODS

1. Type strains

Type strains are as follows: *Streptococcus agalactiae* (KCCM 40417), *Streptococcus mitis* (KCTC 3556), *Streptococcus mutans* (KCTC 3065) and *Streptococcus salivarius* (KCTC 3960).

2. Reagent solutions

1) Citric acid-trisodium phosphate-saline solution (CTS, pH 6.0);0.3g of citric acid monohydrate (SIGMA, USA) and 0.8g of TSP 12 hydrate (SIGMA, USA) were dissolved into 100㎖ of normal saline.
2) TSP-saline solution (TS, pH 8.4); 0.8g of TSP 12 hydrate was dissolved into 100㎖ of normal saline, and pH was controlled by 5N HCl.
3) Citric acid-saline solution (CS, pH 4.6); 0.4g of citric acid monohydrate was dissolved into 100㎖ of normal saline, and pH was controlled by 5N NaOH.
4) Saline control; 0.85% NaCl solution

3. Experimental procedures

Type strains were shaken-cultivate in disposable plastic tissue culture flasks (CORNING, 25㎠, USA) containing brain heart infusion broth (BHI, DIFCO, USA) and three glass beads (SI 5013, φ 7㎜, SiliBead, Germany) at the speed of 70 rpm in shaking incubator (HAN BAEK, SCIENTIFIC CO. KOREA). After eighteen hours shaking incubation, glass beads were picked up with three-pin-pointed pincette and slightly washed with normal saline. Three glass-beads were then put into the four different tubes each with different solutions. In order to give brushing effect that is similar to the brushing of one's teeth, 40㎎ of bits of weighing paper (K-ace, KOREA) clipped into small pieces (2-3㎜) were put into each tube. The tubes were shaken by vortex mixer (GW-92VM, Whasin, Korea) in the velocity of level 10 for 10 minutes except for the non-oral microbe, *Streptococcus agalactiae* (5 minutes). The three samples from each reagent solution were colony-counted by serial agar dilution method, and the average colony counts were calculated. All *Streptococcus spp.* were incubated in 5% CO_2 environment for 48 hours. Experiments were repeated three times for each *Streptococcus* species. The average colony counts of each reagent solution were divided by that of normal saline, and the final anti-sticking effect of each reagent solution was the average value of three experimental results.

RESULTS

The de-adherence degree of citric acid-trisodium phosphate-saline solution (CTS, pH 6.0) against *Streptococcus mutans* came to an average of 12.5 times stronger compared with that of saline control. Trisodium-saline (TS, pH 8.4) showed the average of 7.5 times, and citric acid-saline (CS, pH 4.6) showed 6.0 times compared to the control group (Table 1). The bacterial de-adherence degree against *Streptococcus salivarius* was 7.2, 2.6 and 2.8 times above reagent sequence respectively in comparison with saline control (Table 2). CTS and TS showed 2.4 and 3.4 times of anti-sticking effect on *Streptococcus mitis* respectively, but CS had no anti-sticking effect on this bacteria (Table 3). CTS, TS and CS showed 0.7, 0.6 and 0.6 times on non-oral microbe, *Streptococcus agalactiae*, separately (Table 4). CTS solution has showed the strongest anti-sticking effect on *S. mutans* and *S. salivarius* among three solutions compared with saline control (Fig. 1).

DISCUSSION

It is well-known that citric acid has bacteriostatic effect and are widely used in the food and drug industry. Trisodium phosphate is also chemically classified as GRAS by FDA[1,2] of United States and world-widely used in the food hygiene field. A process using a food grade ortho-phosphate (trisodium phosphate, TSP) to reduce viable *Salmonella* in chicken has been approved by the US Department of Agriculture.[9] Many researchers have examined about the decontamination of pathogenic bacteria like *Listeria monocytogenes*,[3, 4, 5, 8, 11, 13] *E. coli* O157:H7[6], *Salmonella spp*[6, 13, 14]. *Yersina enterocolitica*[8], and *Edwardsiella tarda*[13] in several poultry[4, 7] beef[6], and eggs[8]. They have often examined 1-2% acetic acid[12] or 1-2% lactic acid solution[16] with 2-12% TSP solution and reported excellent meat shelf-life results[15]. According to literature survey, there are no reports that examined the low concentration of citric acid and TSP for the study of oral microbes. Moreover, 2% acid or TSP is too high to apply to oral microbes research. Author devised the experimental model to measure the de-adherence degree against oral *Streptococcus spp.* and examined the effect of TSP or citric acid solution. According to this experimental model, citric acid (0.3 g%), trisodium phosphate (0.8 g%) and saline mixture (CTS, pH 6.0) showed strong anti-sticking effect of 12.5 times on *S. mutans* and of 7.2 times on *S. salivarius* compared with normal saline control. Trisodium phosphate-saline (TS, pH 8.4) showed anti-sticking effect of 7.5 times on *S. mutans* and of 2.6 times on *S. salivarius*, respectively. Citric acid-saline (CS, pH 4.6) showed the effect of 6.0 times on *S. mutans* and of 2.8 times on *S. salivarius* each. These results suspect that citric acid-trisodium mixture has synergistic anti-sticking effect on both oral streptococci. The anti-sticking effect on *S. mitis* was 2.4 times in CTS and 3.4 times in TS. There was, however, no effect (0.3 times) in CS in three solutions on non-oral microbe, *S. agalactiae* (Fig.1). Citric acid and TSP are not harmful to the human health. The de-adherence effects on *S. mutans, S. salivarius* and *S. mitis* by our experimental model are also excellent due to the lower concentration level than the level used in the food hygiene field. Giese et al. reported that TSP removes contaminated bacteria from the surface of chicken by the elimination of thin fat layer on chicken surface and has the indirect anti-bacterial effect due to the chelation of essential metallic ions for the growth of bacteria[10]. Ray ect. reported that organic acids have bacteriostatic effect on Gram negative bacteria due to low pH and dissociated or undissociated acid molecules[17]. It is assumed that citric acid and TSP may affect the stickiness of glycocalyx of oral *Streptococcus* species. CTS mixture showed the strongest anti-sticking effect on oral *Streptococci*. Author suggests that CTS mixture (pH 6.0) can be applied to the development of a new conceptive toothpaste to prevent dental caries or subacute endocarditis.

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Table 1. The de-adherence degree against S. mutans by solutions

Reagent solution (pH)	Multiples of deadherence[a]			Average
CTS (6.0)[b]	13.6	12.0	11.9	12.5
TS (8.4)[c]	10.3	8.7	3.4	7.5
CS (4.6)[d]	11.2	2.9	4.0	6.0

[a] The values that colony counts of each reagent are divided by that of saline control.

[b] CTS; citric acid-triphosphate-saline

[c] TS; trisodium phosphate-saline

[d] CS; citric acid-saline

Table 2. The de-adherence degree against S. salivarius

Reagent solution	Multiples of de-adherence[a]			Average
CTS[b]	10.3	8.2	3.0	7.2
TS[c]	2.8	2.8	2.3	2.6
CS[d]	3.5	2.8	2.2	2.8

[a] The values that colony counts of each reagent are divided by that of saline control.

[b] CTS; citric acid-triphosphate-saline

[c] TS; trisodium phosphate-saline

[d] CS; citric acid-saline

Table 3. The de-adherence degree against S. mitis

Reagent solution	Multiples of de-adherence[a]			Average
CTS[b]	1.7	3.6	1.9	2.4
TS[c]	1.7	5.5	3.0	3.4
CS[d]	0.1	0.5	0.4	0.3

[a] The values that colony counts of each reagent are divided by that of saline control.

[b] CTS; citric acid-triphosphate-saline

[c] TS; trisodium phosphate-saline

[d] CS; citric acid-saline

Table 4. The de-adherence degree against S. agalactiae

Reagent solution	Multiples of de-adherence[a]			Average
CTS[b]	1.3	0.3	0.4	0.7
TS[c]	0.2	0.7	0.8	0.6
CS[d]	0.5	0.8	0.5	0.6

[a] The values that colony counts of each reagent are divided by that of saline control.

[b] CTS; citric acid-triphosphate-saline

[c] TS; trisodium phosphate-saline

[d] CS; citric acid-saline

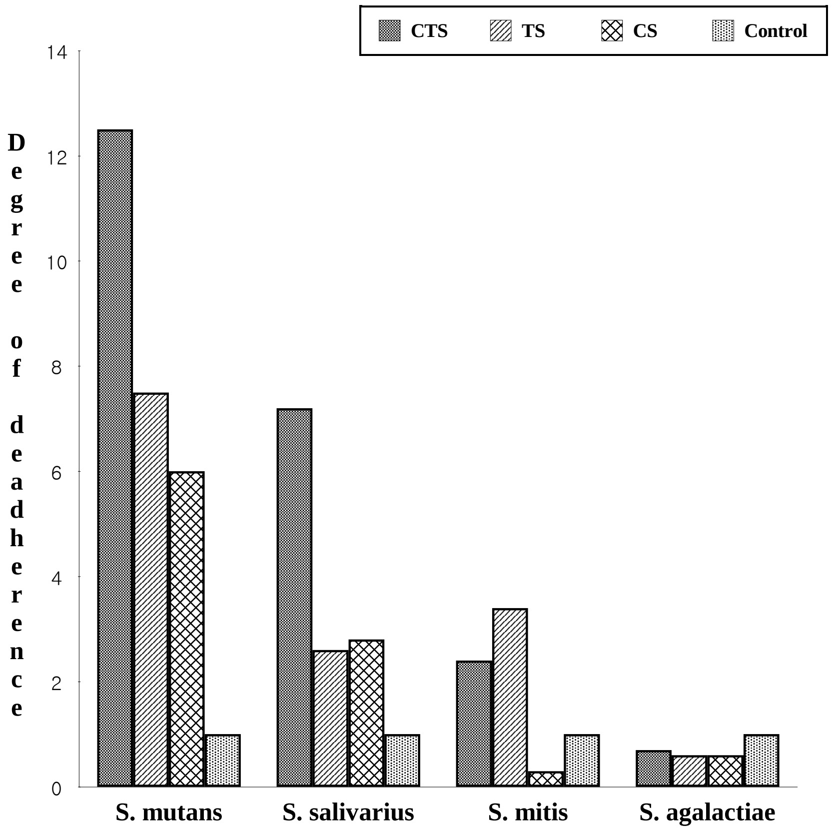

Fig. 1. The comparison of de-adherence by reagent solutions

Figure 1. The comparison of de-adherence by reagent solutions

저작물 이용 허락서

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논문제목	한글 : 구연산과 제3 인산나트륨 혼합액의 구강 내 사슬알균에 대한 강력한 항 부착 능력				
	영문 : The Anti-sticking Effect of Mixture of Trisodium Phosphate and Citric Acid on Oral *Streptococcus* species.				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집과 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물 이용의 허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속 대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의(0) 반대( )

2008년 7월 일

저작자: 조형훈 (인)

조선대학교 총장 귀하