Characterization and application of Aml on the cariogenic streptococci

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[Introduction]

Autolysins are bacterial peptidoglycan hydrolases (PGHs) that digest peptidoglycan of their own when cells are placed in unfavorable conditions. Because of their antimicrobial efficacy and selectivity, autolysins have been suggested as potential alternatives to antibiotics. An autolysin produced by *Streptococcus mutans* has been previously identified and named as automutanolysin (Aml). Aml selectively lyses *S. mutans* and *S. sobrinus* that are major pathogens of dental caries. This striking feature of Aml suggested a possibility to apply Aml for dental caries prevention. Here we present a new approach to control dental caries by specifically eradicating the cariogenic pathogens without affecting the resident oral flora.

To establish safe and effective use of Aml for future clinical testing, I evaluated the Aml activity towards *S. mutans* in vivo and in vitro.

1) **P1670 enhanced the lytic activity of Aml in vitro**

Previous study suggested that a combination of Aml and a non-ionic detergent Triton X-100 could increase the activity of Aml in vitro. But Triton X-100 can't be used for oral administration. Based on the knowledge that non-ionic detergent can improve the activity of Aml, we searched for compounds and found sucrose fatty acid esters as possible candidates nonionic surfactants. Because of their nontoxicity, they have been approved as the common ingredient in food preparations. Our study demonstrated that sucrose fatty acid esters show the ability to improve the activity of Aml in vitro and the P1670, a palmitic acid sugar ester, showed the strongest activity to enhance Aml activity. My study has consistently suggested the presence of P1670 strongly enhance the lytic activity of Aml against *S. mutans* in vitro.

Therefore, we used combination of p1670 and Aml to test the in vivo activity of Aml against *S. mutans* infection.

2) **Aml inhibitory effects on *S. mutans* infection in vivo**

To examine the effectiveness of Aml in vivo, an animal experiment was carried out using murine *S. mutans* infection model. Sprague-Dawley rats at 15 days of age were randomly divided into four groups: group A, negative control group without *S. mutans* MT8148R (a streptomycin-resistant strain) inoculation and Aml treatment; group B, positive control group with *S. mutans* MT8148R inoculation for 1 week; group C, inoculation of *S. mutans* MT8148R and Aml for 1 week followed by Aml treatment alone for 2 weeks; group D, inoculation of *S. mutans* MT8148R for 1 week followed by Aml treatment for 2 weeks. After 4 weeks, experiments were terminated, and plaque and saliva were collected by swab for
colony count every week, plated onto MSB\textsuperscript{st} agar to count numbers of \textit{S. mutans} MT8148R colonies, and BHI agar to count CFU of whole oral floras. The numbers of \textit{S. mutans} MT8148R was presented as percentage of the total oral floras for statistical analysis.

Start of Aml treatment at the period of \textit{S. mutans} inoculation (group C) resulted in a significant suppression of \textit{S. mutans} colonization when compared to the \textit{S. mutans} inoculated positive control group B or to the group D that Aml was administered 1 week later of \textit{S. mutans} inoculation. On the other hand, there was no statistical difference for the CFU of whole floras among 4 groups. These results suggest that Aml is selectively acting on \textit{S. mutans in vivo} and is able to gain access to \textit{S. mutans} in the early stage of colonization and exerts lytic activity. This might delay maturation of biofilm formation and suppress or even decrease CFU of \textit{S. mutans in vivo}.

3) **Combined use of Aml and dextranase for biofilm-forming \textit{S. mutans}**

\textit{In vivo} experiment suggested that maturation of biofilm formation may be an obstacle for Aml treatment. Dextranase (1,6-\alpha-D-Glucan-6-glucanohydrolase) has been shown to inhibit glucan formation and used in clinic. We therefore studied combination effect of Aml and dextranase \textit{in vitro}. Combination of Aml, dextranase and P1670 decreased the aggregation and optical density of \textit{S. mutans} in broth culture in the presence of sucrose.

4) **Establishment of LPS-free Aml using \textit{B. choshinensis} expression system**

Recombinant Aml used for \textit{in vivo} and \textit{in vitro} experiments was prepared from an \textit{E. coli} expression system. But preparation of recombinant protein using \textit{E. coli} expression system cannot exclude a possible contamination of endotoxin, lipopolysaccharides (LPS) in the preparation. In this study, I established a recombinant Aml preparation system using \textit{Brevibacillus choshinensis} expression system and purified Aml as a secreted form in the culture supernatant. Moreover, the recombinant Aml showed the comparable activity to \textit{E. coli}-expressed recombinant Aml.

[Discussion]

Aml in the presence of P1670 showed inhibitory effects on \textit{S. mutans} colonization \textit{in vivo}, but didn’t affect other oral floras. Combination of Aml and dextranase showed a synergistic effect on \textit{S. mutans} killing \textit{in vitro}. This combination might be a next regiment for \textit{in vivo} study.

Furthermore, the secretory production of recombinant Aml using \textit{B. choshinensis} expression system established a convenient way for large scale Aml preparation without the contamination of LPS. Our study further suggests the possibility of clinical application of Aml for dental caries prevention.