Association of specific *Streptococcus mutans* strains with development of non-alcoholic steatohepatitis

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Objective

Non-alcoholic steatohepatitis (NASH) is a fatty liver condition that leads to liver inflammation. In our previous studies, collagen-binding protein (Cnm)- and 190-kDa cell surface protein antigen (PA)-positive *Streptococcus mutans* strains were frequently detected in NASH patients. Here, the involvement of these specific *S. mutans* strains in development of NASH was assessed using a mouse model.

Methods

C57BL/6J mice (6-week-old males) were fed a high-fat diet for four weeks, then a Cnm- and PA-positive (Cnm^+/PA^+) *S. mutans* KT3 strain isolated from a patient with severe NASH, was intravenously injected via the jugular vein. After 12 weeks, the mice were euthanized and blood samples collected, and whole body, extirpated liver tissue, and visceral fat weight measurements performed. Hematoxylin-eosin (HE) staining was

used to assess inflammatory cell infiltration and fat deposition in the liver, and Masson's-trichrome (MT) staining to evaluate fibrosis. In another experiment, KT3, KT4 (Cnm^-/PA^+), KT2 (Cnm^+/PA^-), and KT44 (Cnm^-/PA^-) strains were injected. After 1 or 3 hours, liver, visceral fat, and subcutaneous fat specimens were collected and placed into Mitis-salivarius agar plates including bacitracin, and the number of bacterial colonies was counted.

Results

Liver weight as well as serum levels of ALT, AST, TC, and LDL was significantly increased after injection of the KT3 strain as compared to the control group without infection ($P<0.05$). In liver tissues, HE staining revealed marked lipid accumulation with lobular inflammation and MT staining showed marked fibrosis. All strains of *S. mutans* were detected in all specimens from injected mice, though the number was significantly higher in liver specimens obtained at 1 hour after infection of with the KT3 strain.

Conclusion

These findings suggest that when Cnm- and PA-positive *S. mutans* organisms in the

bloodstream reach a liver with fat accumulation, NASH aggravation is induced.

(291/300 words max)

***Key words**: non-alcoholic steatohepatitis, *Streptococcus mutans*, collagen-binding protein, protein antigen

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Collagen-binding Protein Pathogenicity in Rat Model of IgA Nephropathy

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Objectives: We previously reported that administration of collagen-binding protein (Cnm)-positive *S. mutans* in a rat model caused IgA nephropathy-like nephritis in the jugular vein. The present study investigated involvement of Cnm in development of IgAN-like nephritis in a jugular vein model using a Cnm deletion mutant strain and its complement strain, as well as recombinant Cnm (rCnm).

Methods: An *S. mutans* SN74 strain, that with Cnm deletion (CND), a complement strain (Comp), and rCnm were used, with each administered through the jugular vein of individual Sprague-Dawley rats (4-week-old males). After 45 days, the rats were euthanized, then urine and blood samples collected, and kidneys extracted. Evaluations of renal tissue pathology in the kidneys were performed using periodic acid-schiff (PAS) staining and fluorescent immunostaining with IgA, C3 antibodies.

Results: There were no significant differences in major serum measurements or urinary protein levels among the groups. On the other hand, the hematuria positivity rate was significantly higher in the SN74 group as compared with the CND ($P<0.05$) and control (PBS) ($P<0.01$) groups. Histopathological analyses of sections stained with PAS revealed prominent proliferation of mesangial cells and mesangial matrix in rats in the

SN74, Comp, and rCnm groups. Mesangial proliferation scores were significantly higher in the SN74 and Comp groups as compared to the PBS and CND groups, while that score for the rCnm protein group was significantly higher than that of the CND group. Furthermore, immunohistochemical analyses using IgA- and C3-specific antibodies showed prominent positive reactions in mesangial regions of rats inoculated with SN74, Comp, or rCnm protein.

Conclusions: These results led us to speculate that Cnm protein on the surface of *S. mutans*, but not the bacterium itself, is an important virulence factor involved in *S. mutans*-related IgAN.

【Key words】

1. *Streptococcus mutans* 2. Collagen-binding protein 3. IgA nephropathy
4. Recombinant Cnm