Streptococcus mutans adhesion to Liver affects Non-alcoholic Steatohepatitis Development

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Objectives

Non-alcoholic steatohepatitis (NASH) is steatohepatitis with inflammation. A recent study noted frequent detection of collagen-binding protein (Cnm)- and 190-kDa cell surface protein antigen (PA)-positive *Streptococcus mutans* strains in NASH patients. Furthermore, those strains were found to invade the bloodstream and exacerbate the disease in animal models. The present study examined localization of *S. mutans* isolated from the oral cavity of a NASH patient in a mouse model and the association with disease development.

Methods

Six-week old C57BL/6J mice were fed a high-fat diet for four weeks, then (Cnm+/PA+) *S. mutans* KT3 strains isolated from a severe NASH patient were intravenously administered under general anesthesia. Euthanasia at 12 weeks after administration and conventional clinical evaluations of NASH were performed, while liver tissues were histopathologically evaluated. To examine bacteria localization, KT3, KT4 (Cnm-/PA+), and KT2 (Cnm+/PA-) strains were also injected. After one or three hours, liver, visceral, and subcutaneous fat specimens were collected and inoculated into Mitis-salivarius agar plates containing bacitracin, and then the number of bacterial colonies was counted. Results were compared with control group mice that received PBS.

Results

Liver weight and serum levels of ALT, AST, TC, and LDL were significantly of KT3 group were significantly higher in the KT3 group ($P<0.05$). Histopathologic findings in KT3 group livers also showed prominent lipid accumulation with inflammatory cell infiltration and fibrosis. All *S. mutans* strains were detected in all specimens from injected mice, though the number was significantly higher in liver specimens obtained at one hour

after infection with the KT3 strain (P<0.01). Additionally, the numbers of KT3 strains in visceral and subcutaneous fat after one hour were greater as compared to the other strains.

Conclusion

These findings suggest that Cnm- and PA-positive *S. mutans* that invade blood may reach the liver and adipose tissue, and become involved with development of NASH.

(296/300 words max)

Summary: *Streptococcus mutans* localization in adipose tissue and liver has effects on pathogenesis in a NASH mouse model

Key words

1. Non-alcoholic steatohepatitis
2. *Streptococcus mutans*
3. Collagen-binding protein
4. Protein antigen

IgA adherence to *Streptococcus mutans* Cnm for IgA Nephropathy Onset

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Objective

Streptococcus mutans, the major causative bacteria of dental caries, has several surface proteins related to its pathogenicity including Cnm, which shows collagen binding protein (Cnm). The most common form of chronic glomerulonephritis in pediatric patients is IgA nephropathy (IgAN), defined immunohistologically as a disease associated with IgA deposited in the renal glomerular mesangial region. We have conducted examinations of IgAN onset focused on Cnm-positive *S. mutans* organisms, with findings in a previous study indicating that administration of *S. mutans* with Cnm caused transient induction of IgAN-like lesions in rats. In the present study, adherence

of Cnm to IgA, and invasion by and deposition of Cnm in kidney tissue were examined.

Methods

Human IgA (IgA1) was coated onto 96-well microplates, then recombinant Cnm (rCnm) was added. Washing with phosphate buffer saline was performed, then anti-rabbit-Cnm followed by an alkaline phosphatase-conjugated rabbit immunoglobulin antibody were added to all wells. After washing, a color detection solution was added and OD_{430} measured. Next, rCnm was blotted onto a polyvinylidene difluoride membrane using a Bio-Dot® microfiltration apparatus and reacted with IgA1. The procedure noted above was again performed. Following visualization, membrane intensities were determined using ImageJ®. Additionally, biotin-labeled rCnm was administered into the jugular vein of specific pathogen-free 4-week-old male Sprague-Dawley rats, then the kidneys were evaluated following immunofluorescent staining with IgA antibodies.

Results

The rate of binding of IgA1 to rCnm was significantly increased in a dose-dependent manner in both experiments ($P<0.05$). Immunofluorescence staining showed IgA deposition in the glomerular mesangial region, with biotin-labeled rCnm observed in the same region as IgA deposition.

Conclusion

The present results indicate that IgAN-like nephritis is induced by binding of Cnm to IgA and its deposition in renal glomeruli. It is considered that Cnm is likely an important factor related to onset of IgAN.

Summary: Collagen-binding protein Cnm of *Streptococcus mutans* binds IgA and induces IgA nephropathy-like nephritis.

Keywords: *Streptococcus mutans*, IgA nephropathy, Cnm-protein, Biotin, human IgA

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