

SHORT COMMUNICATION

Heterogeneous Glycopeptides from Gastric Mucin Inhibit Dental Decay in Rats: A Pilot Study

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ABSTRACT

The aim of this pilot study was to investigate whether the application of sufficient amounts of soluble adhesin receptor analogues in the oral cavity could effectively inhibit the lectin mediated binding of *Strep. mutans*, and consequently the development of dental caries *in vivo*. Germ free rats were orally infected with *Strep. mutans* and were fed a high cariogenic diet. The control-group was given sterilized tap water. The test group received a sterilized solution of hydrolyzed gastric mucin. The test group developed significantly less carious lesions on approximal surfaces. Our data revealed that a continuous supplementation of glycopeptides from gastric mucins via drinking water is a potential inhibitor of dental decay in gnotobiotic rats.

Keywords: Microbial adhesion, Carbohydrates, Dental caries, Gnotobiotic rats.

INTRODUCTION

The widespread use of fluorides in dentistry is believed to be mainly responsible for the caries decline in the most western countries. It has led to a polarization of the disease (Pettersson and Bratthall, 1996),⁷ i.e. most of the caries lesions can be found in a relatively small group of highly caries susceptible individuals. Those individuals need additional preventive strategies. The colonization of teeth by cariogenic *Strep. mutans* involves the interaction of bacterial lectin-like adhesins with the carbohydrate-portion of glycoconjugates immobilized on the tooth surface (Cisar et al 1997; Gibbons 1989; Kolenbrander et al 1993).^{1,2,4} *In vivo* and *in vitro* studies showed that soluble glycans in secretions can prevent infections, e.g. in the urogenital tract by inhibiting the lectin mediated adhesion of *E. coli* to urothelium cells (Svanborg Edén et al 1982; Zopf and Roth 1996).^{10,12} Recently, we showed that quantitative reduction of a putative *Strep. mutans* glycan receptor in children is associated with an increased caries risk (Seemann et al 2001).⁸ *In vivo* and *in vitro* studies have shown that soluble glycans in secretions can prevent lectin mediated adhesion of pathogens and subsequent infections, if present in excess quantities (Strömberg et al 1996; Svanborg Edén et al 1982; Zopf and Roth 1996).^{9,10,12} Therefore, the application of sufficient amounts of potential adhesin receptor analogues within the oral cavity should effectively inhibit the lectin mediated binding of *Strep. mutans*, and consequently the development of dental caries *in vivo*.

MATERIALS AND METHODS

The study was conducted on 2 litters of caries active germ-free Sprague Dawley rats. Each litter consisted of 10 pups. On day 21 after birth the pups were weaned. Males and females were equally distributed between test (n=10) and control group (n = 10). Both groups were fed a high cariogenic diet (modified diet C 1065, Altromin, Lage, Germany with 56% saccharose and Geval Instant Protein, Lederle, Gosport, UK). The control-group was given sterilized tap water. The test group was offered a sterilized solution of glycopeptides (mol. weight lower than 10 kDa) obtained by exhaustive proteolysis with proteinase K of a porcine mucin (Saliva medac, Hamburg, Germany). Both fluids were offered ad libitum. On day 22 and day 29, all rats were orally infected with a cariogenic *Strep. mutans* strain (ATCC 25175). On day 56, all rats were sacrificed and weighed. Caries scores were obtained by the evaluation of sagittal serial sections from the lower jaw molars with respect to the extent of demineralization in fissures and on approximal surfaces after staining. A carious lesion was classified as enamel lesion when present only in the enamel, as initial dentine lesion when present in the outer dentine and as established dentine lesion, when present in the inner dentine (Keyes 1958; König 1966).^{3,5}

RESULTS

There was no significant difference between the test and control animals according to the body-weight (control: 267gm ± 61 gm; test: 275 gm ± 42 gm) indicating that consumption of the

Table 1: Mean number of caries lesions (\pm standard deviation)

	Test (n = 10)	Control (n = 10)	Significance (t-test)
n (male/female)	5/5	5/5	
Enamel-lesions			
• Fissures	7.0 (\pm 3.6)	8.3 (\pm 3.3)	ns ^a
• Approximal areas	0.8 (\pm 1.4)	3.5 (\pm 3.2)	p < 0.05
Initial dentine-lesions			
• Fissures	3.1 (\pm 3.8)	6.1 (\pm 4.1)	p < 0.05
• Approximal areas	0.3 (\pm 0.9)	3.4 (\pm 3.2)	p < 0.01
Established dentine-lesions			
• Fissures	0.8 (\pm 1.3)	3.5 (\pm 3.2)	p < 0.05
• Approximal areas	0.2 (\pm 0.6)	2.2 (\pm 2.8)	p < 0.05

^anot significant

cariogenic diet did not differ between the groups. In Table 1, the caries data are summarized. Compared with the control, the test group showed significantly less initial dentine lesions and established dentine lesions. The caries inhibiting effect of glycopeptides was more obvious for approximal surfaces than for fissures. This might be due to better access of glycopeptide-resolution to these surfaces than to fissure grooves, which represent an anatomically retention form for microorganisms and food. Further studies are in progress to specify the “active” structural component in the heterogeneous glycoconjugate mixture considering that oral bacteria are believed to possess more than one type of adhesin (Marsh and Bradshaw 1995).⁶

DISCUSSION

With these data, we report for the first time that *in vivo* a continuous supply of anti-adhesive complex glycans via drinking water results in a decreased development of carious lesions in rats monocolonized with a cariogenic Strep. mutans strain. These findings indicate that application of soluble glycopeptides might be an effective anticariogenic supplement and could also have high impact for the development of new treatment strategies for other bacteria derived oral diseases. However, it has been shown that non-mutans-streptococci (MS) play an important role in the caries process (for review see Takahashi and Nyvad, 2008).¹¹ Their adhesins are one reason for their importance at the initial stage of plaque formation. The adhesins of MS do not attach as efficiently to the acquired pellicle. Further studies should focus on Non-MS testing their capability to inhibit dental decay.

ACKNOWLEDGMENT

This work was supported by a grant from the Charité Berlin. We like to thank, Alfred Russ, Dieter Czwick and Thomas Kersten for their technical help.

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