INHIBITORY EFFECT OF MUTASTEIN ON THE SYNTHESIS OF ARTIFICIAL, DENTAL PLAQUE BY *STREPTOCOCCUS MUTANS*

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It is believed to be a significant step for the induction of dental caries that cariogenic *Streptococcus mutans* synthesizes adhesive, insoluble glucan from sucrose and adheres to tooth surfaces to form dental plaque in the oral cavity[1]. This is supported by the fact that mutant strains of *S. mutans* defective in adhesive glucan synthesis lack cariogenicity[1]. Thus, inhibition of the synthesis of the adhesive, insoluble glucan is expected to be an effective means for preventing plaque formation and caries development.

Mutastein, a heat-stable glycoprotein isolated from the culture filtrate of *Aspergillus terreus*, was identified as a specific inhibitor of glucan synthesis[2]. This substance suppresses significantly plaque formation and caries development in rats[3], and is now used commercially as a protective agent against caries development in humans. Two glucosyltransferases (GTase) are known to be involved in the glucan synthesis by *S. mutans*, one (GTase-S) that catalyzes soluble glucan synthesis and one (GTase-I) that converts soluble glucan to the adhesive, insoluble glucan[4]. Mutastein was reported to inhibit the latter enzyme of several strains of *S. mutans*[2,5].

In the present experiments, the inhibitory effect of mutastein on glucan synthesis in the cell-free enzyme system of *S. mutans* 6715 and the formation of artificial, dental plaque by growing cells of this strain were studied.

Mutastein was isolated from the culture filtrate of *A. terreus* as described previously[2]. Briefly, the culture filtrate was adjusted to pH 3.5 with HCl and the resultant precipitate was collected by centrifugation. The precipitate was submitted to ammonium sulfate precipitation and chromatography of hydroxyapatite and Toyopearl HW-65. Cell-free extracts of *S. mutans* 6715, containing both GTase-S and GTase-I[2], synthesized adhesive, insoluble glucan along with small amounts of nonadhesive, insoluble glucan and soluble glucan (Table 1). Mutastein inhibited adhesive, insoluble glucan synthesis by 45% at a concentration of 0.1 μg/ml and almost completely at 0.4 μg/ml. On the other hand, synthesis of both nonadhesive, insoluble glucan and soluble glucan was markedly stimulated by mutastein; the activation was 3-fold for the former and 10-fold for the latter at a mutastein concentration of 0.4 μg/ml (Table 1). The data suggest that mutastein inhibited specifically the conversion of soluble glucan to adhesive, insoluble glucan.

Artificial dental plaque was formed according to the method of MCCABE *et al*[6]. *S. mutans*

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Table 1. Effect of mutastein on the glucan synthesis by cell-free extract of *Streptococcus mutans* 6715.

Mutastein (μg/ml)	Glucan synthesis (mg/ml)		
	Adhesive insoluble	Nonadhesive insoluble	Soluble
0	0.940	0.090	0.061
0.05	0.710	0.155	0.185
0.10	0.513	0.253	0.305
0.20	0.170	0.273	0.610
0.40	0.002	0.255	0.585

Fig. 1. Effect of mutastein on the formation of artificial plaque on Nichrome-steel wires by *Streptococcus mutans* 6715.

Experimental conditions were described in the text.

1; Control, 2; 15 μg/ml mutastein, 3; 50 μg/ml mutastein.

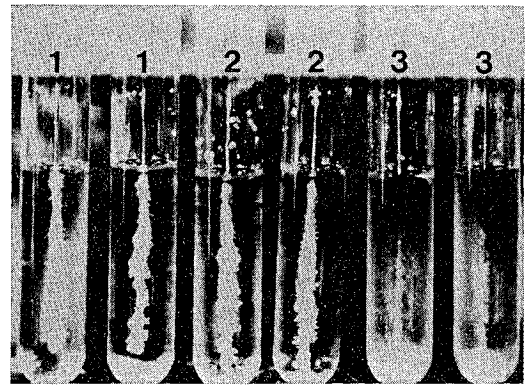
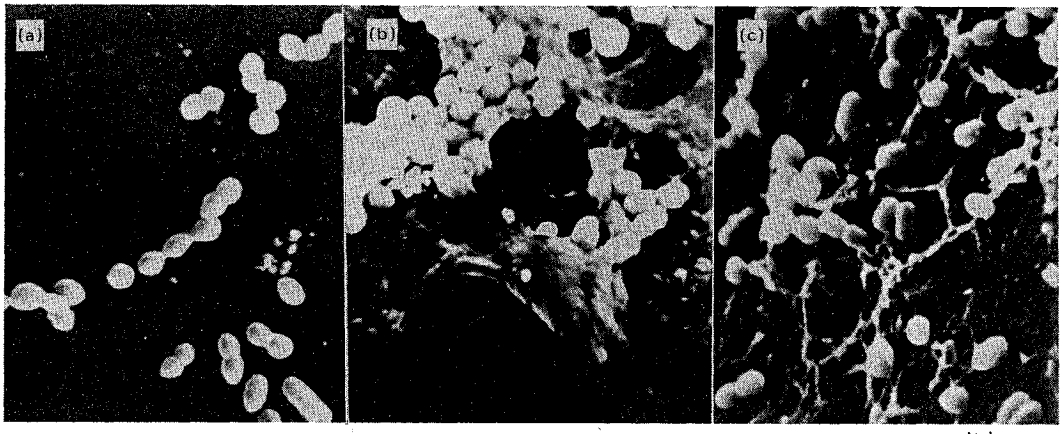


Fig. 2. Scanning electronmicroscopic observation of *Streptococcus mutans* 6715 cells adherent to glass surface.

S. mutans 6715 was grown in BHI broth (a), in BHI broth supplemented with 1% sucrose (b) or 1% sucrose plus 50 μg/ml of mutastein (c). After 18 hours of incubation at 37°C, cell plaques formed on glass surface were submitted to a scanning electronmicroscope (Hitachi S-430).



6715 was grown in 10 ml of brain heart infusion (BHI) broth (Nissui Medical Co., Tokyo) supplemented with 5% sucrose in a 16×150 mm test tube. A sterile 20-gauge Nichrome-steel wire, 15 cm long, mounted in a rubber stopper was suspended in the culture medium. After incubation at 37°C for 24 hours, the wire was transferred to a test tube containing fresh culture medium inoculated with *S. mutans* 6715. This procedure was repeated 9 times. The wire was then dipped in fresh medium (with no inoculation) and incubated at 37°C for 5 days. Under these conditions, adhesion of cells (plaque) was observed on the wire (Fig. 1-1, control). These cells were tightly bound to the wire and not released from the wire by gentle shaking. In a parallel study when 15 μg/ml of mutastein was added to the culture medium, a similar adhesive plaque developed (Fig. 1-2). However, the plaque formed in the presence of 50 μg/ml of mutastein was loose and easily released from the wire by gentle shaking (Fig. 1-3). These results support that mutastein inhibits synthesis of the adhesive, insoluble glucan involved in the formation of dental plaque.

No significant cell plaque and aggregation were formed when *S. mutans* 6715 was grown in BHI broth (not supplemented with sucrose) (Fig. 2a). When cells were grown in BHI broth supplemented with 1% sucrose, however, aggregation of cells, possibly mediated by adhesive, insoluble glucan, was observed (Fig. 2b). This aggregation of cells were strongly inhibited by 50 μg/ml of mutastein (Fig. 2c). The adhesive glucan mass was also reduced by mutastein.

These experiments further support our previous observation[5] that mutastein specifically inhibits synthesis of adhesive, insoluble glucan by *S. mutans* and is effective in the reduction of artificial, dental plaque formation. It is interesting that cell plaques formed in the presence of mutastein were structurally loose and easily released from the wire, as compared to those in the control experiment. These results as well as electronmicroscopic observations strongly support a significant role of adhesive, insoluble glucan in the plaque formation and inhibitory effect of mutastein in this process.

References

1) HAMADA, S. & H. D. SLADE: Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. 44: 331~384, 1980
2) ENDO, A.; O. HAYASHIDA & S. MURAKAWA: Mutastein, a new inhibitor of adhesive-insoluble glucan synthesis by glucosyltransferases of *Streptococcus mutans*. J. Antibiotics 36: 203~207, 1983
3) NAKAMURA, Y.; H. KUWASHIMA, T. MASUHARA & A. ENDO: Inhibitory effect of mutastein on caries development in rats inoculated with

Streptococcus mutans 6715. Jpn. J. Oral Biol. 27: 603~610, 1985

4) FUKUSHIMA, K.; R. MOTODA, K. TAKADA & T. IKEDA: Resolution of *Streptococcus mutans* glucosyltransferases into two components essential to water-insoluble glucan synthesis. FEBS Lett. 128: 213~216, 1981

5) KOGA, T.; S. HAMADA, S. MURAKAWA & A. ENDO: Effect of glucosyltransferase inhibitor on glucan synthesis and cellular adherence of *Streptococcus mutans*. Infect. Immun. 38: 882~886, 1982

6) MCCABE, R. M.; P. H. KEYES & A. HOWELL, Jr.: An *in vitro* method for assessing the plaque forming ability of oral bacteria. Arch. Oral Biol. 12: 1653~1656, 1967