

## Laboratory and Epidemiology Communications

### Clinical Study of Mutans Streptococci Using 3DS and Monoclonal Antibodies

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Communicated by Nobuhiro Hanada

(Accepted March 21, 2001)

*Streptococcus mutans* has been implicated as the primary causative organism of dental caries (1). *S. mutans* produces surface protein antigen (Pac) (2) for the purpose of initial attachment and produces glucosyltransferase (GTF)(3) for the synthesis of water-insoluble glucans (WIG). These two substances contribute to the cariogenic properties of *S. mutans* in the oral cavity (4). Recently, an anti-Pac monoclonal antibody (KH5, MAb) recognizing an alanine-rich repeating region (residues 365 to 377) was identified as an antibody that inhibits the attachment of *S. mutans* to tooth surfaces in rats (5,6). While an anti-GTF monoclonal antibody (p-126, MAb) inhibiting WIG synthesis by GTF-I (7) has been discovered. KH5 and p-126 MAb may also be useful for protection against dental caries caused by *S. mutans*.

Ma et al. have demonstrated that passive immunization using specific antibodies against *S. mutans* is potentially effective for protection against dental caries (8,9). Passive immunization is safe and specific. In this study, we investigated the clinical efficacy of anti-Pac and anti-GTF MAb in eliminating *S. mutans* from the oral cavity. Clinical application of anti-microbial agents to tooth surfaces using a dental drug delivery system (3DS) has been reported (10). In 3DS, an individual tray that covers the whole dentition is used as a drug retainer. A tray that fits over the tooth surfaces is useful for delivering small but sufficient amounts of anti-bacterial drugs. It is easy to manipulate and maintains high concentrations of MAbs in direct contact with tooth surfaces. Thus, MAbs are not diluted by saliva, and only the bacteria on the tooth surface, but not those on the oral mucosa, are exposed to MAbs. Before the application of 3DS, dental biofilms were removed by means of professional mechanical tooth cleaning

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(PMTC). After application of 0.2% chlorhexidine (CHX), an effective antiseptic in dentistry (11), the mixed MABs were applied to the tooth surface also by 3DS. The subjects consisted of five healthy human volunteers (age range: 47-60 years old).

The subjects were treated first with phosphate buffered saline (PBS) as a control and then with mixed MABs (12.6 mg/

person) as a trial. Saliva samples were taken before and after the 3DS with CHX and then at 20, 30, 50, and 70 days after the PBS or MAB treatment. Saliva secreted over a period of 3 min, while being stimulated by biting paraffin gum, was collected into ice-chilled sterile bottles. The saliva was transferred via a cotton swab into the transport medium (0.4% agar, 0.15% thioglycolate/PBS) in sterile tubes (SEEDSWAB No. 1; Eiken Chemical Co., LTD, Tokyo).

The sample was further transferred with a cotton swab into 4 ml of Brain Heart Infusion (BHI) broth. The BHI broth containing the saliva samples was sonicated by an ultrasonic dispersion apparatus (60 W power output) for 10 sec, and poured onto blood agar plates, and Mitis-Salivarius agar plates with (MSB) or without 0.02 M bacitracin (MS) in a volume of 50  $\mu$ l using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo). After anaerobic culture for 48 h, total bacterial colonies on the blood agar, total Streptococci colonies on the MS plates and *S. mutans* colonies on the MSB plates were counted. *S. mutans* could be identified by their characteristic appearance.

In three subjects, A, D and E, *S. mutans* was eliminated by means of CHX treatment and failed to re-colonize during the period of control and trial studies. In subjects B and C, mixed MABs treatment for 70 days significantly reduced *S. mutans* in total streptococci to 38% and 39%, respectively, of the values before the treatment (i.e., after treatment with PBS) (Fig. 1). However, there were no significant changes in the number of total bacteria and total streptococci observed after the MAB treatment (Fig. 2). These results suggest that the application of mixed MABs by means of 3DS minimally disturbs the oral flora and is useful for eliminating *S. mutans* in the oral cavity.

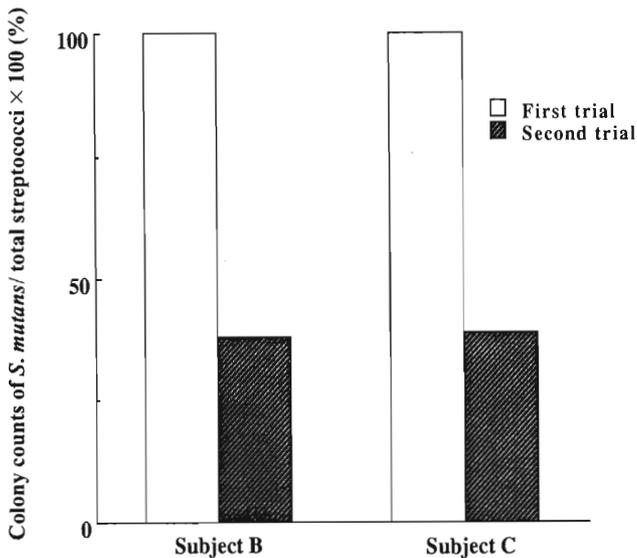


Fig. 1. Effect of the application of mixed MABs by 3DS on *S. mutans* population in total streptococci. The figure presents the ratio (%) of *S. mutans* colony counts to total streptococci colony counts in subjects B and C. The data for subjects A, D, and E were omitted because *S. mutans* were eliminated via CHX pre-treatment.

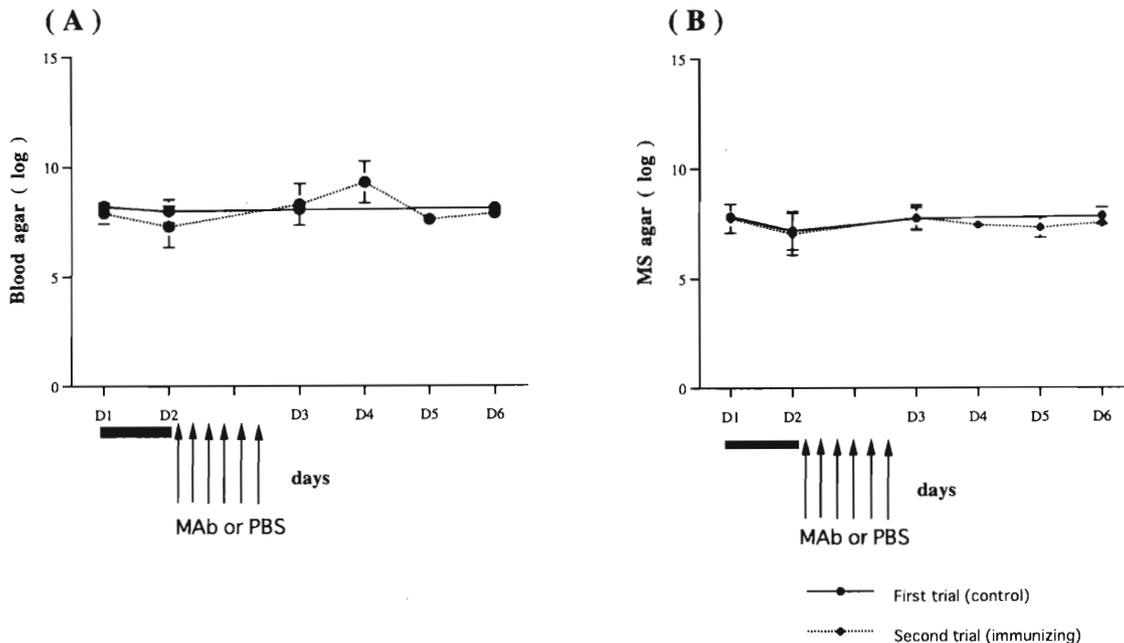


Fig. 2. Effect of the application of mixed MABs by 3DS on the colony counts of total bacteria and streptococci.

A: Colony counts of total bacteria in stimulated saliva.

B: Colony counts of total streptococci in stimulated saliva.

— : control study. ····· : trial. ■ : period of CHX treatment.

Saliva samples were taken before (D1) and after (D2) the 3DS with CHX and at 20 (D3), 30 (D4), 50 (D5) and 70 (D6) days after the PBS or MAB treatment.

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