Betaine reduces the irritating effect of sodium lauryl sulfate on human oral mucosa in vivo

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Our aim was to evaluate whether betaine has a protective effect during exposure of the human oral mucosa in vivo to sodium lauryl sulfate (SLS) or cocoamidopropylbetaine (CAPB) as measured with a multifrequency electrical impedance spectrometer (EI). Both detergents were used at the concentration of 2.0% w/v with and without 4.0% w/v betaine in distilled water in 20 volunteers, and 0.5% and 1.0% w/v SLS combined with 4.0% w/v betaine in 5 volunteers. EI measurements were taken before application of the test solutions, after their removal, and every 15 min up to 45 min. Both 0.5% and 1% SLS solutions showed a significant reduction in 3 of the 4 indices, indicating mucosal irritation after the 15-min exposure ($P < 0.05$), whereas 2% SLS did so in all 4 indices ($P < 0.001$). Betaine had no effect on the detergent-induced decline with either the 2% or the 0.5% SLS solutions. However, when combined with the 1% SLS solution, betaine significantly ($P < 0.05$) reduced mucosal irritation by abolishing decreases in indices MIX (magnitude index) and IMIX (imaginary part index) and lowering it for PIX (phase index). The 2% CAPB solution showed a significant ($P < 0.05$) reduction in all 4 indices after the 15-min exposure, but the effect was significantly weaker than that of 2% SLS ($P < 0.05$). Betaine did not reduce the irritating effect of 2% CAPB. These findings can be used in the development of less irritating products for oral health care.
□ *Betaine; cocoamidopropylbetaine; electrical impedance; oral mucosa; sodium lauryl sulfate*

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The detergents in oral health care products, such as sodium lauryl sulfate (SLS) and cocoamidopropylbetaine (CAPB), can cause mild to strong changes in the human oral mucosa (1). Detergents, however, are commonly believed to enhance toothpaste penetration into approximal spaces between the teeth, thus prolonging toothpaste-retention on the teeth; the fluoride compounds therefore have a longer time to be in contact with the dentition. Detergents also produce foam when brushing and improve the plaque-removing effect of the toothpaste even though their antibacterial effect is minor (2).

SLS is the most widely used synthetic detergent in dentifrices and its concentration usually ranges from 0.5% to 2.0% (2). It is an anionic detergent that emulsifies the plaque deposits and dissolves lipophilic substances in toothpastes or mouthwashes (3). SLS is often used to induce experimental contact dermatitis as a side effect, as it causes inflammation and desquamation of the oral mucosa and increases permeability to oil-soluble compounds, especially by denaturizing the oral mucin layer (4, 5). SLS has been shown to cause irritative effects on oral mucosa (4–7). More detailed studies have shown that SLS causes mucosal desquamation at least at high concentrations, or in direct cap splint application, in concentrations ranging from 1.0% to 1.5% (6–9). An elevation in microcirculation after application of SLS on human oral mucosa and skin has been reported earlier (7, 10, 11). SLS has also been connected with recurrent aphthous ulcers (12), but studies not showing this association have also been published (13).

Amyloalkylobetaines are considered to be less irritating detergents in dental care products than SLS (14). For example, CAPB is used in dental applications at the concentration of 2.45%. CAPB has earlier been tested in cap splint application at concentrations of 0.64%, 1.27%, and 1.90% (6), and in a 6-week testing at a concentration of 1.9% (15). A significantly lower irritating effect compared to that of SLS was observed in both tests (6, 15).

Betaine (trimethylglycine, glycinebetaine), a natural product derived from sugar beet, has been studied as a protector of living cells against harmful substances (16). It diminishes the skin-irritating properties of various ingredients used in cosmetics; for example, detergents (17). Betaine, when added to the SLS-containing toothpaste, reduces the irritating effects of SLS and relieves subjective symptoms of dry mouth (18). Furthermore, betaine is an osmoprotectant in bacteria, plants, and animals, and it protects cell components against harsh conditions in vitro (19). It has also been suggested that betaine is suitable for use in osmotic stress experiments in vitro as a direct probe to quantify changes in hydration of protein surface in biopolymer processes (20). Betaine is presently used in animal foods, plant protection and fertilizing agents, and in nucleic acid and antibiotic drug production as well as in the cosmetic industry.

The aim of the present study was to evaluate whether

Fig. 1. The specially made plastic clip with the 18-mm paper disk and aluminum cap.

betaine has any protective effect during short-term exposure of the oral mucosa to two different irritating detergents, SLS and CAPB. The electrical impedance method we used has been shown to be capable of detecting subtle, SLS-induced changes in the oral mucosa (21).

Materials and methods

Subjects

Twenty non-smoking volunteers were recruited from the personnel of the Institute of Dentistry, Turku, Finland (16 women and 4 men; age range 22 to 54 years (mean 31.9 ± 6.0 years)). The flow rate of paraffin-stimulated whole saliva varied between 0.1 and 2.5 ml/min (mean 1.5 ± 0.6 ml/min). All volunteers had a macroscopically normal oral mucosa. None used any medication, but 5 reported allergies to antibiotics or hay, and 1 had atopic eczema. The subjects were informed not to eat or drink anything in the hour prior to each test. The Ethics Committee of the Faculty of Medicine, University of Turku, approved the study and all volunteers signed an informed consent form.

Test substances

SLS (SLS, Texapon K 12, Henkel, KOGA, Düsseldorf, Germany) was used at concentrations of 0.5%, 1.0%, and 2.0% w/v with and without 4.0% w/v concentration of betaine (Betafin BP, Finnfeeds, Naantali, Finland) in distilled water. Cocoamidopropylbetaine (CAPB; Tegobetain CK D, Th. Goldsmith AG, Essen, Germany) was used at the concentration of 2.0%, with and without 4.0% of betaine in distilled water. The test solutions were adjusted to pH 6.0, as the protective ability of betaine is highest at this pH. Distilled water was used as a control.

Study design

The study was a double-blind, randomized clinical experiment. A paper disk, impregnated with 300 μl of the test solution, was put in the 18-mm Finn Chamber (Epitest Ltd Oy, Rannankoukku 22, FI-04300 Tuusula, Finland), which is an aluminum cap covered with non-allergenic plastic film. The paper disk with the aluminum chamber was placed inside both cheeks to the lower border of the occlusion plane of the buccal mucosa, approximately 1.5 cm from the corner of the mouth just beside the lower molars, and kept in place for 15 min with a specially made plastic clip (Fig. 1). Nicander et al. (22) have used this design earlier.

The test subjects went through the procedure 3 times: firstly with distilled water on both cheeks as a control substance. Secondly and thirdly, 2 different randomly chosen test solutions on both cheeks, 2 at the same time (2% SLS or CAPB with or without 4% betaine). Five of the test subjects took part in a further study with lower concentrations of SLS (1.0 and 0.5%) in the presence and absence of 4% betaine. The test solutions were prepared each week and the test was carried out in a double-blind manner. The washout period between different test solution exposures was at least 6 h. Before exposure to a new solution it was ascertained that the visible alterations in the mucosa and the measured impedance values had returned to baseline.

Evaluation method

For impedance measurements, we used the electrical impedance spectrometer (SciBase AB, Huddinge, Sweden) described by Nicander et al. (22). Readings were taken before application of the test solutions, immediately after their removal, and then every 15 min for the next 45 min. The same investigator carried out all the examinations (IR).

The electrical impedance instrument records the magnitude and phase at 31 logarithmically distributed frequencies between 1 kHz and 1 MHz, in a volume under the probe. This volume can be selected to include contributions, stepwise, from 5 different depths (21). In the present study, impedance spectrum was recorded at all 5 depth settings at each test site. The different surfaces behave differently, and this is why the different depths cannot be described as exact millimeter values. The exact millimeter depths for impedance settings are not known. The instrument, which is controlled by a computer program, measures impedance automatically and stores the results in a database within a few seconds after the probe has made good contact with the mucosa. The electrical impedance spectrum was analysed in terms of 4 indices emphasizing different aspects of the impedance properties of the tissue (22). For detection of irritant reactions a set of 4 indices based on 20 kHz and 500 kHz has been devised, emphasizing different aspects of the impedance properties of the tissues. They are: 1) Magnitude index, $\mathrm{MIX} = \mathrm{abs}(Z_{20\mathrm{kHz}})/\mathrm{abs}(Z_{500\mathrm{kHz}})$; 2) phase index, $\mathrm{PIX} = \arg(Z_{20\mathrm{kHz}}) - \arg(Z_{500\mathrm{kHz}})$; 3) real part index, $\mathrm{RIX} = \mathrm{Re}(Z_{20\mathrm{kHz}})/\mathrm{abs}(Z_{500\mathrm{kHz}})$; and 4) imaginary part index, $\mathrm{IMIX} = \mathrm{Im}(Z_{20\mathrm{kHz}})/\mathrm{abs}(Z_{500\mathrm{kHz}})$, where abs is the magnitude, arg the phase angle in degrees, and Re and Im the real and imaginary parts of the impedance Z at the frequencies indicated. The quotients MIX, RIX, and IMIX are dimensionless. For PIX, the phase index, we

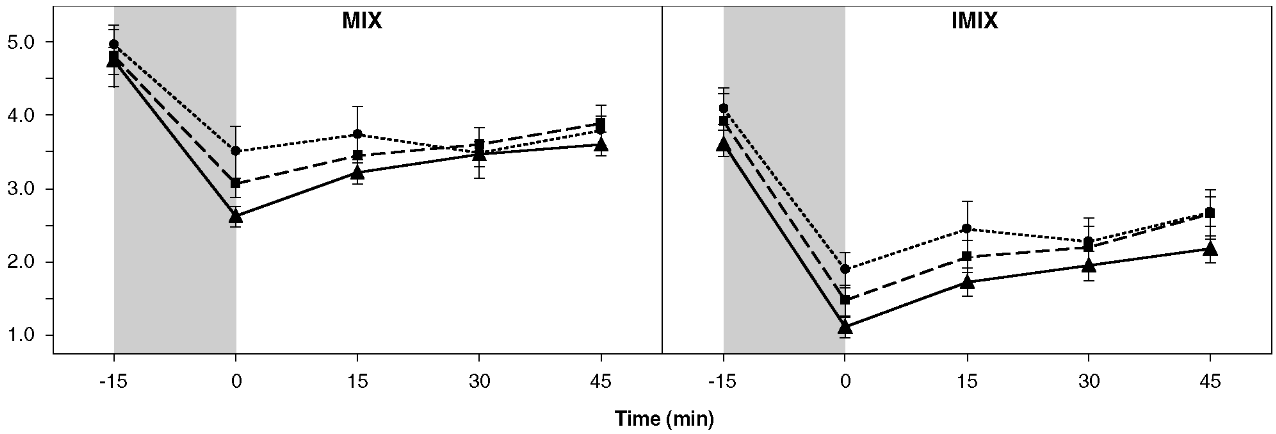

Fig. 2. The decrease induced by 0.5% SLS (●), 1% SLS (■), and 2% SLS (▲) in the MIX and IMIX indices at depth 1 of oral mucosa (mean ± SEM). Grey area indicates the exposure time. For statistical significance, see text. For 2% SLS, $n = 20$; for 0.5% and 1% SLS, $n = 5$.

chose the unit degrees because the index has an angular dimension. The electrical impedance method shows the changes on skin and oral mucosa, but cannot be directly interpreted in exact changes in mucosal cell structures or intercellular spaces and junctions. We can only see and measure the irritative effect on impedance indices, we cannot tell exactly what causes the effects.

Statistics

The statistical evaluations of the variables recoded with electrical impedance were conducted using parametric repeated measurements analysis of variance (RANOVA), n being the same. Only in the case of significance were the pairwise comparisons within the groups carried out with Student's t tests. The level of statistical significance was set at <0.05. For statistics, we used the SPSS 10.0 software program.

Results

All the detergent-containing solutions caused visual roughness in oral mucosa, whereas the control solution, distilled water, did not affect the mucosal appearance at all.

The mean values and standard errors of the mean (SEM) deviations were calculated for the 4 impedance indices (MIX, PIX, RIX, and IMIX) at depth settings 1 and 5 at all test sites. Distilled water did not cause any changes in any of the 4 impedance indices during the 15-min exposure, and both sides of the buccal mucosa behaved similarly. The 0.5% and 1% SLS solutions gave a significant ($P < 0.05$) decline after the 15-min exposure in the MIX, PIX, and IMIX indices at depths 1 and 5. No effect was seen for RIX (Fig. 2, MIX and IMIX). The 2% SLS solution caused a significant decline in all 4 EI indices at depths 1 and 5 ($P < 0.001$). The 0.5% and 1% SLS solutions showed no significant difference compared to baseline index values at the 45-min follow-up time. For the 2% SLS solution, most indices had not returned to baseline by that time ($P < 0.05$).

The 2% CAPB solution caused a significantly lower ($P < 0.05$) decrease in all 4 EI indices compared to the 2% SLS solution at depths 1 and 5 (Fig. 3), the decrease being

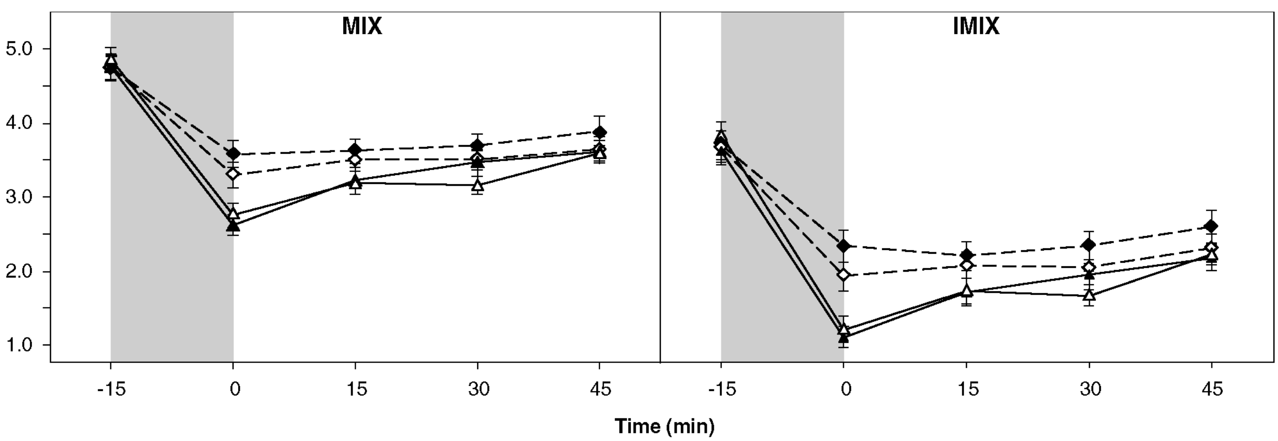

Fig. 3. The decrease in indices MIX and IMIX at depth 1 on mucosal exposure of 2% CAPB (◆), 2% SLS (▲), 2% CAPB + 4% betaine (◊) and 2% SLS + 4% betaine (△) (mean ± SEM). Grey area indicates the exposure time. For statistical significance, see text ($n = 20$).

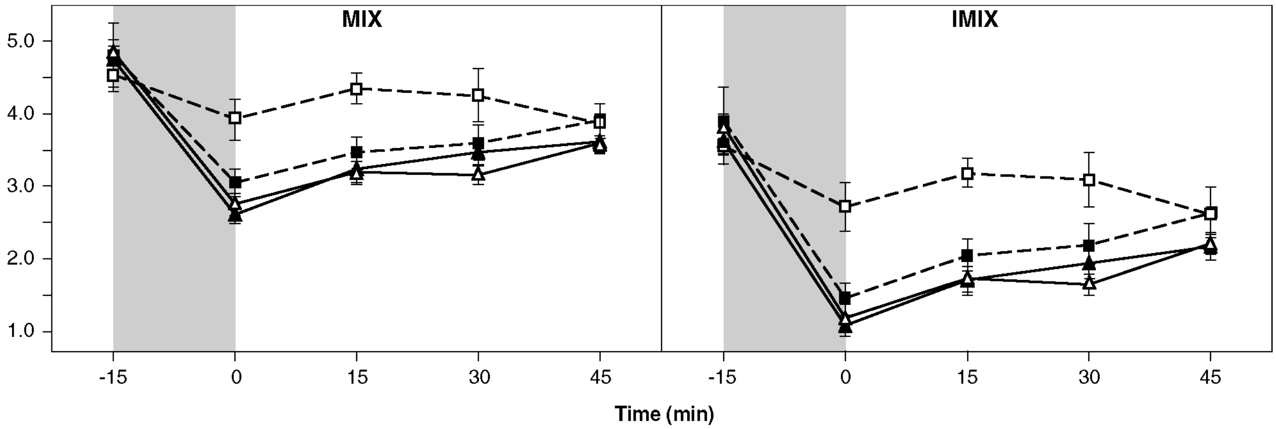

Fig. 4. The decrease induced by 1% SLS (■), 2% SLS (▲), 1% SLS + 4% betaine (□), and 2% SLS + 4% betaine (△) in the indices MIX and IMIX at depth 1 (mean ± SEM). Grey area indicates the exposure time. For statistical significance, see text. For 2% SLS ± betaine, $n = 20$; for 1% SLS ± betaine, $n = 5$.

similar to that of 0.5% SLS. The indices did not return to baseline values for CAPB during the 45-min follow-up.

Betaine significantly ($P < 0.05$) decreased the irritating effect of 1% SLS on the mucosa, and the effect was seen even with the small number of test subjects ($n = 5$). Betaine abolished the effect of 1% SLS as measured with indices MIX and IMIX, and lowered it for PIX ($P < 0.001$), while no effect was observed in RIX (Fig. 4, MIX and IMIX). Betaine did not alter the immediate mucosal responses to the 0.5% or 2% SLS or the 2% CAPB solutions, nor did it hasten their return to baseline values measured by electrical impedance, except for 0.5% SLS, which returned to baseline at 30 min (Figs 3 and 4).

Discussion

Our earlier study (18) suggested that betaine-containing SLS toothpaste could reduce subjective sensations of dry mouth. The xerostomic patients preferred the betaine-containing toothpaste to the placebo toothpaste representing ordinary commercially available SLS toothpastes (18). Our present findings revealed that 4% betaine significantly reduced the mucosa-irritating effect of the 1% SLS solution, but not that of the other 2 SLS concentrations, 0.5% and 2%. In the study carried out with the dry mouth subjects (18), the paste contained 2% SLS and brushing time was approximately 3 min, while in the present study the exposure time was 15 min. The SLS clearance from the oral cavity has earlier been reported to be 96% in 2 min when used in a rinse, compared to 86% in 10 min when used in a toothpaste (23). Thus, the actual exposure time of the oral mucosa to SLS did not differ very much between the present study with the solution and the previous one (18) with the toothpaste. However, toothpaste contains several other ingredients in addition to detergent and betaine, and these components could have modified the response of the oral mucosa to the exposure. The results of the irritation-relieving effect of 4% betaine on 1% SLS in solution are supported by our recent observations in which human skin was exposed to SLS and betaine for 24 h (24).

In the present study, we used the same experimental design as Nicander et al. (22). The earlier study demonstrated that the effects of 15-min exposure of the oral mucosa to a 2% SLS solution were detectable and characterized by the 4 electrical impedance indices at both depth 1 and depth 5. The beneficial effect of 4% betaine on the immediate irritating effects of 1% SLS was seen at both depth settings 1 and 5 for all electrical impedance indices except RIX, the conductivity index.

In the literature, a more frequent use of amidobetaines is suggested (6, 14, 15). Although the effects of amidobetaines are generally considered to be less irritating than those of SLS, we found that the effect of 2% CAPB was detectable both at the mucosal surface and at deeper mucosal layers, and that baseline values were not reached during the 45-min follow-up. The CAPB response was similar to the SLS response, as measured by the 4 electrical impedance indices, but weaker than the SLS response. Our results are in accordance with Herlofson & Barkvoll (6), who reported a dose-independent result in a cap-splint test with artificial amidobetaine CAPB.

Besides visual inspection, which is the usual way of evaluating reactions in the oral mucosa, some bioengineering approaches to a non-invasive objective evaluation have been suggested, such as laser Doppler flowmetry (25) and electrical impedance (21) as used in the present study. Traditional methods used to study skin, such as transepidermal water loss, cannot be used to study the moist oral mucosa. The electrical impedance technique has been shown to be suitable for detection of reactions, particularly in the invisible or barely visible range of both the skin and the oral mucosa (21). Furthermore, a good correlation between skin changes induced by different concentrations of SLS and changes in electrical impedance has been found (26). The histological changes, such as edema, parakeratosis, and leucocyte infiltration after SLS exposure, are observed to be quantitatively dose-dependent

(26). The mainly spongiotic edema, predominant in the basal layer of the epidermis, can be the reason for changes in electrical impedance indices after the exposure (26). The previous skin measurements (26) do not directly correlate with the non-keratinized oral lining mucosa, which is more readily affected with the various surfactants.

In previous studies, a set of 4 impedance indices was introduced, representing different aspects of electrical impedance. The results indicate that these indices were capable of differentiating between the reaction patterns caused by 3 different irritant substances, also verified by histological examination (27). Our study also confirms the applicability of electrical impedance in studies of subtle changes in human oral mucosa.

The oral mucosa is exposed to detergents in toothpastes and oral health care products daily, even several times a day. Clinically, this means that even minor irritative effects can cause problems in the long run, especially in those who have dry or sensitive oral mucosa.

Betaine had no effect on the immediate mucosal effects of 0.5% and 2% SLS or 2% CAPB, but abolished the irritating effect of 1% SLS. In our study, CAPB caused a measurable mucosal irritation corresponding to the effect of 0.5% SLS, even though CAPB has been suggested to be less irritative than SLS. Our study demonstrates that SLS-containing dentifrice formulas should be tested for their mucosa-irritating effects. Furthermore, the optimal betaine concentration for oral health care products should be explored.

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