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The effect of sodium lauryl sulphate and triclosan on hamster cheek pouch mucosa

JOHAN H. BAERT*, RIKKE J. VEYS†, KRISTIEN AMPE‡ AND JAN A. DE BOEVER†

**Interdisciplinary Research Center, Laboratory for Histology, Katholieke Universiteit Leuven, Campus Kortrijk, Belgium; Departments of †Periodontology and ‡Removable Prosthodontics, School of Dentistry, Universiteit Gent, Belgium*

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Summary. It has recently been shown that triclosan protects the human skin from the inflammation that may be caused by exposure to sodium lauryl sulphate (SLS). The aim of the present study was to examine whether triclosan can protect the hamster cheek pouch mucosa from the irritation caused by exposure to SLS. After four daily applications of a paste containing SLS, the epithelium of the hamster cheek pouch showed consistently prominent structural changes, especially basal hyperplasia, acanthosis, hypergranulosis, and hyperkeratosis. Identical morphological changes were also observed after applications of a paste containing SLS together with triclosan. In contrast, after applications of a paste containing triclosan alone, the cheek pouch mucosa revealed a histological structure essentially similar to the non-treated control mucosa. From these results, we may conclude that SLS, but not triclosan, irritates the hamster cheek pouch epithelium. Moreover, triclosan does not protect the cheek pouch mucosa against structural changes induced by SLS. It must be taken into account that triclosan does not always offer protection against the side-effects of SLS.

Keywords: sodium lauryl sulphate, triclosan, irritation, mucous membrane, hamster cheek pouch

The anionic detergent sodium lauryl sulphate (SLS) is the most widely used cleansing agent in cosmetics and dentifrices. It is generally known as a bactericidal substance for several bacterial species (Giertsen *et al.* 1989). SLS is also known to cause dermatitis when applied to human skin, probably due to destruction of lipid-containing semipermeable membrane systems in the cornified surface layer (Beradesca *et al.* 1990). In the oral cavity, there exist several indications that SLS may interact with soft and hard tissues (for review, see Veys *et al.* 1992): it may increase the permeability of the oral mucosa and denature the protective mucin layer, which provides natural protection and increased resistance. It is well established that SLS may cause inflammation and desquamation of the oral mucosa (Herlofson & Barkvoll 1993) and a burning pain (Waaler *et al.* 1993).

In our previous study (Veys *et al.* 1994), we have shown prominent changes in the histological structure of hamster cheek pouch epithelium after topical application of SLS (in dentifrice or in paste), including hyperkeratosis, acanthosis and varying degrees of basal

Correspondence: Professor Dr Johan Baert, Interdisciplinary Research Center, Laboratory for Histology, Katholieke Universiteit Leuven, Campus Kortrijk, E. Sabbelaan 53, B-8500 Kortrijk, Belgium.

hyperplasia, all resulting in a statistically significant increase in thickness of the epithelium.

Triclosan is a lipid-soluble broad spectrum antibacterial agent, often used in soap and cosmetics. As indicated by Lindhe (1990), triclosan has also been found to be a useful antibacterial agent for incorporation into oral products because of its effect on bacteria found in the oral cavity, compatibility with oral product ingredients and a long history of use in consumer products. It is included in dentifrices and mouthwashes to reduce plaque and calculus formation, to prevent caries, and to improve gingival health (Saxton *et al.* 1987; Lindhe 1990; Cubells *et al.* 1991; Mankodi *et al.* 1992). It is suspected that the anti-gingivitis effect of triclosan may at least in part be explained by an anti-inflammatory effect (Barkvoll & Rölla 1994). Based on several pre-clinical and clinical studies, triclosan can be considered safe for use in dentifrices and mouth-rinse products (for review, see DeSalva *et al.* 1989).

In their pilot study, Waaler *et al.* (1993) observed that the presence of triclosan in mouthwashes appeared to reduce the untoward side-effects of SLS, such as desquamation and burning sensation. The reason for this is not clear. However, it is well established that triclosan penetrates the oral mucosa and skin easily (Gilbert & Williams 1987). In a recent study, Barkvoll and Rölla (1994) showed that triclosan protects the human skin from the inflammation that may be caused by exposure to SLS. The aim of the present study was to examine whether triclosan can protect the hamster cheek pouch mucosa from the irritation caused by exposure to SLS (Veys *et al.* 1994). The hamster cheek pouch is generally accepted as a suitable model for testing the irritant effect of substances on the mucous membrane (Cutright *et al.* 1974; Harsanyi *et al.* 1991).

Materials and methods

We used 15 male adult Syrian golden hamsters. The following three pastes were applied on cheek pouch mucosa: (1) a paste containing 1.5% SLS (provided by ICI Holland BV; content of active anionic matter: 89–91%), (2) a paste containing 1.5% SLS and 0.3% triclosan (provided by Ciba Geigy BV; extinction of 25 mg/l ethanol: 0.45–0.49), and (3) a paste containing 0.3% triclosan, each with alumina hydrate and glycerol/sorbitol as a vehicle. Each paste was applied on the mucosa of the medial wall of the cheek pouch in five hamsters. The animals were slightly anaesthetized with chloroform. Using a cotton bud, the pastes were gently rubbed on the pulled out mucosa of the right cheek pouch for 15 seconds. An area of approximately 1 cm^2, distal to the anterior vein, was carefully rubbed. In each animal, four applications were performed at intervals of 24 hours. On the fifth day, specimens of the treated mucosa were carefully excised from the anaesthetized animals. Specimens of the non-treated mucosa from the left cheek pouch were excised as control sites. The animals were killed by cervical dislocation.

The mucosal specimens were stretched and pinned down, with the connective tissue surface downwards, on cork sheets to keep them flat during processing. The tissues were fixed by immersion in 10% neutral buffered formalin for either 72 hours at room temperature or 4 hours at 4°C. After fixation, the specimens were trimmed to approximately 8×8 mm, to ensure that the samples are representative of painted mucosa, then cut into two pieces and routinely processed for paraffin embedding. Tissue sections were cut at 5 μm perpendicular to the epithelial surface, stained with a routine trichrome staining, and examined with a Zeiss Photomicroscope III. Fifteen tissue sections from each cheek pouch were studied. Between sample sections there was a space of at least 250 μm.

Results

All epithelial lining of the control cheek pouches was a thin, regular, orthokeratinized stratified squamous epithelium which lacked rete ridges (Figure 1). The epithelium–connective tissue junction was rather flat. The basal epithelial layer consisted of a single row of cuboidal to low columnar cells with round to oval nuclei. The spinous layer consisted of one or two layers of larger and somewhat flattened cells. The granular layer consisted of one or two layers of highly flattened cells with a more or less pyknotic nucleus and with fine keratohyalin granules in their cytoplasm. The superficial layer was composed of tightly packed cornified cells.

Cheek pouch mucosa painted with paste containing triclosan alone (Figure 2) consistently revealed a histological structure essentially similar to the non-treated control tissue.

The cheek pouch mucosa treated with the paste containing SLS (Figures 3 and 5) and the mucosa treated with the paste containing SLS and triclosan (Figures 4 and 6–8) consistently revealed marked but identical structural changes. The thickness of the epithelium was strikingly increased (Figures 3–8). The basal layer showed varying degrees of hyperplasia. The interface between the basal layer and the underlying connective tissue was more or less irregular. Mitotic figures in the basal layer were increased compared with normal controls. In some areas, the hyperplastic basal layer was

composed of palisaded cells (Figures 3 and 4). Epithelial buds, which extented for a variable distance into the connective tissue, were clearly observed, and they varied in size and shape (Figures 5 and 7). The epithelial cells of the epithelial buds had mostly a basaloid appearance, with large nuclei and little cytoplasm. The spinous layer was obviously enlarged (acanthosis), and composed of three to five layers of more voluminous cells, with a relatively large pale nucleus containing a prominent nucleolus. The granular layer revealed hypergranulosis and was characterized by large cells containing coarse keratohyalin granules (Figures 2 and 3) and a pale, round to ovoid nucleus, often with an obvious nucleolus. The superficial layer was notably thickened and revealed orthokeratotic hyperkeratosis. Usually, the cornified layer was not homogeneously stained (Figures 3–6). Pyknotic nuclei were sometimes found in the lower part of this layer (Figure 7). At the basal surface of the cornified layer, spine-like protrusions into the granular layer were found (Figures 4 and 6). Occasionally, at the exposed epithelial surface, dome-shaped elevations of the superficial layer showing parakeratosis were apparent (Figure 8). On the other hand, in the epithelium we found no loss of cell polarity, no abnormal superficially placed mitoses, and no signs of dyskeratosis. Signs of inflammation were never observed in the subepithelial connective tissue.

Discussion

In previous studies, it was observed that the pain caused by 1.5% SLS in mouth-rinses was apparently eliminated or decreased by the addition of 0.3% triclosan (Waaler *et al.* 1993), and that 0.3% triclosan added to a 1% SLS solution protects the human skin against dermatitis (Barkvoll & Rölla 1994). Therefore, the initial aim of the present study was to investigate whether 0.3% triclosan can protect the hamster cheek pouch against chemical irritations caused by a paste containing 1.5% SLS (Veys *et al.* 1994).

The histological structure of the cheek pouch mucosa painted with a paste containing triclosan alone was identical to the structure of the unpainted control cheek pouch, and in agreement with the structure previously described in normal hamsters (Albright & Listgarten 1962; White & Gohari 1981). Therefore, triclosan seemed not to irritate the epithelial cells, although we may accept that triclosan can penetrate cornified epithelium (Black *et al.* 1975).

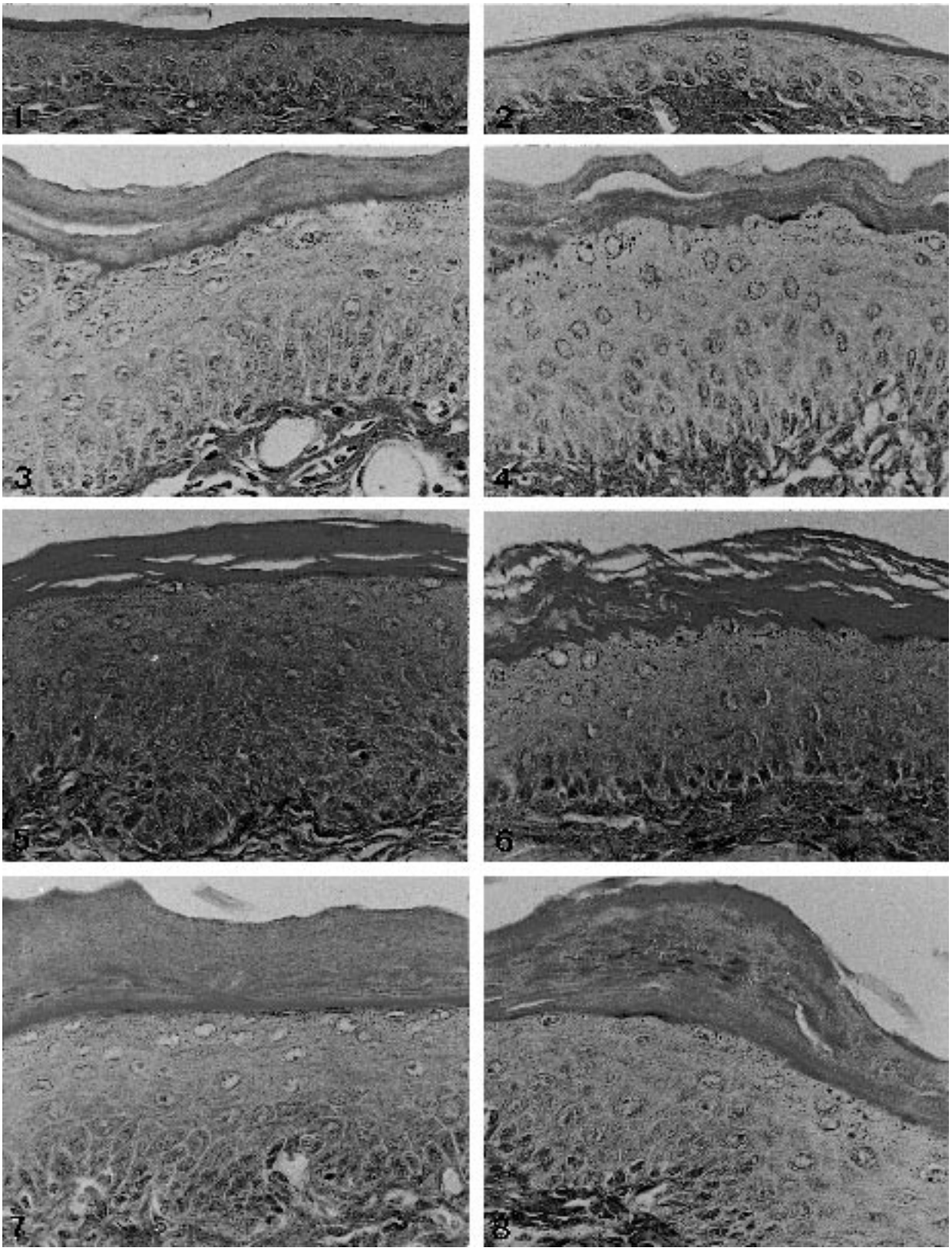
After applications of a paste containing SLS, the cheek pouch mucosa showed consistently marked structural changes, especially varying degrees of basal hyperplasia, irregular acanthosis, hypergranulosis and hyperkeratosis (mostly orthokeratotic hyperkeratosis but occasionally also parakeratotic hyperkeratosis). However, the histological changes seemed not to reveal premalignant changes, and were not accompanied by an inflammatory response in the subepithelial connective tissue. These observations fully confirm the results of our previous study on the possible effects of SLS containing dentifrices on the structure of hamster cheek pouch epithelium (Veys *et al.* 1994).

Applications of the paste containing SLS together with triclosan induced changes in the histological structure of the cheek pouch mucosa which were quite similar to those produced by the paste containing SLS alone. Consequently, we may state that triclosan does not protect the hamster cheek pouch mucosa against the structural changes induced by SLS.

We may accept that SLS most probably has to penetrate the epithelium to cause irritation. Barkvoll and Rölla (1994) suggested that triclosan may exert its protecting effect against SLS-induced dermatitis by binding to the hydrophobic ‘tail’ of SLS molecules and in this way hindering or reducing the penetration of SLS. This suggestion seems not to be supported by the present experiments, as the irritating effect of SLS in combination with triclosan is apparently identified with that of SLS alone. They also suggested that the lipid-soluble triclosan molecules may solubilize in the membranes of the cornified layer and thus stabilize the cells and protect them against the drying out of the tissue by SLS which is probably the cause of dermatitis (Beradesca *et al.* 1990). Upon this suggestion, we may remark that we found no morphological indication for drying out of the cheek pouch tissue by SLS. From the present study we cannot propose any suggestion about the possible effects of triclosan. Even the supposed anti-inflammatory effect of triclosan is not confirmed in the present experiments, as no inflammation is induced by SLS. On the other hand, in further studies it must be taken into account that triclosan does not always offer protection against the side-effects of SLS.

In comparison with our previous paper on the effects of SLS containing dentifrices on hamster cheek pouch mucosa (Veys *et al.* 1994), we here described in more detail some structural changes of the epithelium, because this information may be useful for the understanding of the development of hyperplasia, hypergranulosis, acanthosis and hyperkeratosis.

The epithelial hyperplasia produced after 4 days by SLS was benign and not accompanied by the appearance of structurally dysplastic features. It has been shown that turpentine also has the ability to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produce benign epithelial hyperplasia with hyperkeratosis in hamster cheek pouch, but apparently only after some weeks (Craig & Franklin 1977).

The increased mitotic figures in the basal layer compared with normal controls suggests a relatively high rate of cell proliferation. The basal hyperplasia resulted in an irregular interface between the epithelium and the underlying connective tissue, and epithelial buds, varying in size and shape, were formed. McMillan and Kerr (1990) described scattered rete ridges of varying morphology in small, isolated areas of the normal adult hamster cheek pouch mucosa. However, the examined histological sections contained segments of both the medial and lateral walls of the pouch. Our results relate only to the medial wall of the cheek pouch, and we found no rete ridges in the normal control pouch. As it is known that morphological and physiological differences may occur between the medial and the lateral walls (Scragg & Johnson 1983), the observed epithelial buds may be interpreted as being part of the pathological changes induced by SLS.

The heterogeneous staining of the cornified layer, the spine-like protrusions of this layer into the granular layer, and the appearance of a hyperplastic granular layer containing coarse keratohyalin granules, may all be related to an accelerated keratinization process, caused by SLS and resulting in a statistically significant (Veys *et al.* 1994) increase in thickness of the cornified layer.

It is obvious that the exposure of hamster cheek pouch mucosa to SLS is a suitable experimental model system for analysing chemically induced hyperplasia, acanthosis, hypergranulosis, and hyperkeratosis. Several interesting aspects remain to be further explored, for example, (1) the development of the described structural alterations, (2) the evolution in case of longer exposure to SLS, (3) the reversibility of the structural changes, (4) the specific cytological effects of SLS on the epithelial cells, and (5) the possible interactions of triclosan.

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Figure 1. Control hamster cheek pouch, showing a thin, orthokeratinized stratified squamous epithelium. ×360. **Figure 2.** Epithelium of the hamster cheek pouch treated with triclosan. As in Figure 1, the epithelium is a thin, orthokeratinized stratified squamous epithelium. ×360. **Figure 3.** Epithelium of the hamster cheek pouch treated with SLS. Note the palisaded cells in the hyperplastic basal zone, the coarse keratohyalin granules in the hypertrophic granular layer, and the heterogeneous staining of the thickened cornified layer. ×360. **Figure 4.** Epithelium of the hamster cheek pouch treated with SLS and triclosan. Note the palisaded cells in the basal layer, the coarse keratohyalin granules in the granular layer showing hypergranulosis, and the heterogeneous staining of the thickened cornified layer with spine-like protrusions into the granular layer. ×360. **Figure 5.** Epithelium of the hamster cheek pouch treated with SLS. Note the epithelial buds extending into the connective tissue. The epithelial cells of the buds have mostly a basaloid appearance. ×360. **Figure 6.** Epithelium of the hamster cheek pouch treated with SLS and triclosan. Note the coarse keratohyalin granules in the granular layer showing hypergranulosis, and the heterogeneous staining of the thickened cornified layer with spine-like protrusions into the granular layer. ×360. **Figure 7.** Epithelium of the hamster cheek pouch treated with SLS and triclosan. Note the basal cell hyperplasia, the irregular interface (epithelial buds) between the basal cell layer and the underlying connective tissue, and the highly thickened cornified layer with some pyknotic nuclei in the lower part of this layer. ×360. **Figure 8.** Epithelium of the hamster cheek pouch treated with SLS and triclosan. Note the dome-shaped elevation of the cornified layer showing parakeratosis. ×360.

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