The reversibility of the snuff-induced lesion: an experimental study in the rat

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Snuff lesions were induced in 30 rats. Ten of the snuff-exposed rats were killed immediately after 13 months snuff exposure, as were the 10 control animals. Ten rats were killed 1 month and 10 rats 4 months after the snuff administration had ceased. The rats exposed to snuff for 13 months exhibited hyperplastic, hyperorthokeratotic epithelium with focal mild atypia, focal ulcerations and marked subepithelial fibrosis. These changes were markedly reduced or absent in rats exposed to snuff and killed after a snuff-free interval of 1 or 4 months. Similar differences between the test-groups were seen in the epithelium lining the gingival sulcus of the lower incisors. This area seems to be more sensitive to chemical exposure than the oral mucosa proper as more severe microscopical changes were seen here. Snuff exposure results in the development of a hyperplastic, reactive, reversible lesion of the oral mucosa, suggesting that snuff predominantly has promoting activity when administered for a relatively short interval of time.

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The health consequences of snuff use have been revieved and discussed in detail (1–4). At present, a consensus seems to have been reached acknowledging snuff as an oral carcinogenic agent when used as in North America and western Europe. Snuff-taking is known to induce characteristic lesions in the oral mucous membrane at the site of application of the quid (5–7). These lesions have generally been looked upon as reversible (8). However, this assumption has never been subjected to detailed investigation. This study was designed to evaluate the reversibility of snuff-induced lesions using a well established rat model (9).

Material and methods

Animals

Female Sprague-Dawley rats, 3 months old, (Anticimex, Stockholm, Sweden) were used. Three or four rats were kept in Makrolon cages (No 3 Jacoby, Stockholm, Sweden) and fed a standard pelleted diet (Astra – Ewos AB, Södertälje, Sweden) and water *ad libitum*. The temperature in the animal quarters was kept between 21° and 23°C and the relative humidity was 40%. The light followed daylight rhythm and was never less than 8 h in length.

Animal model

The rats were anaesthetised by intraperitoneal injection of pentobarbitone sodium (60 mg/ml, ACO AB, Solna, Sweden). The dose used was 35 mg/kg body weight. To minimise peroperative bleeding, 0.5 ml of a local anaesthetic (Xylocaine-Adrenaline ® 20 mg/ml + 12.5 µg, Astra Läkemedel Södertälje AB, Sweden) was infiltrated in the submucosa of the lower lip. The mucous membrane of the lip was then excised from the lateral to the lower incisors to 3 mm dorsal to the midline of the lip on both sides in a width of about 2 mm. The 2 wound surfaces thereby created were sutured together. This procedure resulted in a canal covered with mucous membrane. Two teflon plates were applied, one on each side of the lip, with stainless steel wire pulled through the plate-lip-plate. This stabilised and protected the lip during the healing phase for 10 days, after which the plates and sutures were removed (9).

Snuff

A commercially available Swedish brand was used in the study (kindly supplied fresh by Svenska Tobaks AB, Sweden).

Morphological method

Histological examination was performed on the lip, gingival epithelium of the lower incisors (crevicular epithelium), tongue and buccal mucosa. All specimens were fixed in 4% buffered neutral formalin solution and processed and stained by routine methods (H&E, and Weigert van Gieson).

Experimental design

Forty rats were operated on to create the test canal in the lower lip. The animals were then divided into 3 testgoups and one control group. Each group comprised 10 animals. All rats in the test-groups were given approximately 0.2 g of snuff at 8:00 and at 17:00 5 days per week for 13 months. The estimated average length of daily exposure to snuff was 12 h (9). The control animals (n = 10) underwent the

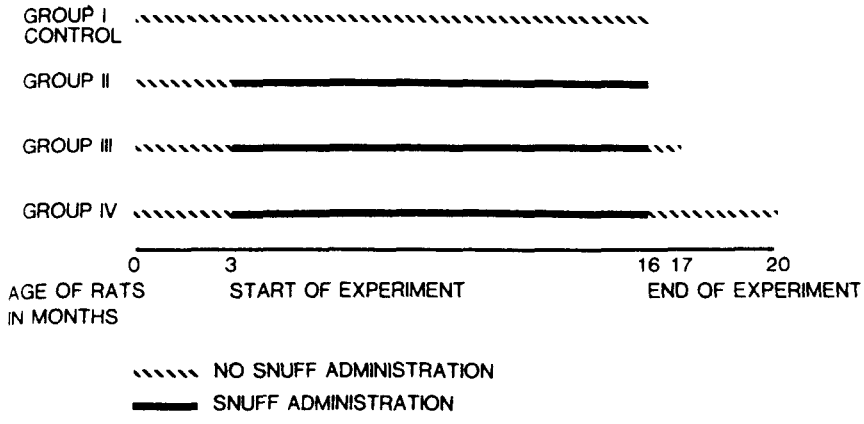

Fig. 1. Experimental design.

same surgical procedure but did not receive snuff. These rats were killed after 13 months. The rats in the first test-group were killed after 13 months of snuff exposure. The rats in the second test-group were killed 1 month after and those in the third test-group 4 months after the snuff administration had ceased. The experimental design is shown in Fig. 1.

Results

The lips of the control animals mostly showed slightly hyperplastic epithelium (90%) * with thickening of both the stratum ganulosum and the stratum spinosum. The surface was covered with a somewhat thickened orthokeratin layer (60%). The lumen of the test canals was partly filled with desquamated keratin (80%, Fig. 2). The rete ridges extending into the connective tissue were only slightly increased and the inflammatory reaction subepithelially in the connective tissue was mild (30%) or absent.

The squamous epithelium of the lips of the first test-group, exposed to snuff for 13 months, exhibited a generalised slight (40%) or moderate hyperplasia (60%). Hyperorthokeratosis was observed in all animals. In certain parts, marked hyperorthokeratosis was seen, while in others a looser type was seen, with focally vacuolated cells extending down into the stratum granulosum (50%). Slightly (80%) or moderatly (20%) acanthotic proliferations with development of marked rete pegs were noted (Fig. 3). The squamous epithelium showed mild focal atypia (40%) as well as focal ulcerations (20%) but the border between the stratum basale and the connective tissue was always well defined. The inflammatory reaction (mostly lymphocytic infiltrates) in the underlying connective tissue was slight (60%) or severe (40%), but above all a prominent fibrosis was noted (100%, Fig. 4).

In the 2 test-groups exposed to snuff and then killed after snuff-free intervals of 1 and 4 months respectively, histopathological changes in the test canals were less prominent. Thus, in comparison with the first test-group, only one rat in each test-group (10%) exhibited ulcerations. The lesions were more atrophic after a snuff-free interval of 1 month (1-month group, 30%) and 4 months (4-month group, 70%), with slight (4-month group, 30%) or no acanthosis (1-month group). The inflammatory reaction was slight (40%) or absent in both these groups (Fig. 5). Mild atypia of the squamous epithelium was only seen in one rat (in the 4-month group). In 60% of the rats with a snuff-free interval of 1 month and in all rats with a 4-month' snuff-free interval, severe subepithelial fibrosis was observed.

The appearance of the squamous epithelium of the tongues of rats killed immediately after 13 months of snuff exposure did not differ from that of the controls.

The squamous epithelium of the tongue and buccal mucosa in rats exposed to snuff for 13 months and killed 1 and 4 months thereafter was mildly hyperplastic (30% 1-month group, 90% 4-month group) and hyperkeratotic (30% 1 month and 70% 4 months) with a mild inflammatory reaction in the subepithelium tissue (30%).

The epithelium of the buccal mucosa was keratinised, with slight or moderate hyperplasia and slight acanthosis in all rats exposed to snuff for 13 months.

Moderate or severe hyperplasia with increased keratinisation was seen in the epithelium lining the gingival sulcus in rats exposed to snuff for 13 months and killed immediately (50%) or after one month (60%, Fig. 6). Focal resorption of the marginal bone plate buccally to the lower incisors was also noted in 2 cases, one in each group. Apart from these findings, the majority of the specimens exhibited atrophy and focal ulcerations (70%) of the gingival sulcus epithelium.

Fig. 2. Light-microscopic appearance of the test canal in a control rat at 13 months. Note the slightly hyperplastic epithelium with orthokeratosis and mild inflammatory reaction in the connective tissue (H&E, ×250).

Fig. 3. Light-microscopic appearance of the test canal in a rat exposed to snuff for 13 months. Note the general moderate hyperplasia with acanthosis and hyperorthokeratosis (H&E, ×250).

* The figures in parentheses represent the percentage of animals in the group exhibiting the particular histologic feature described.

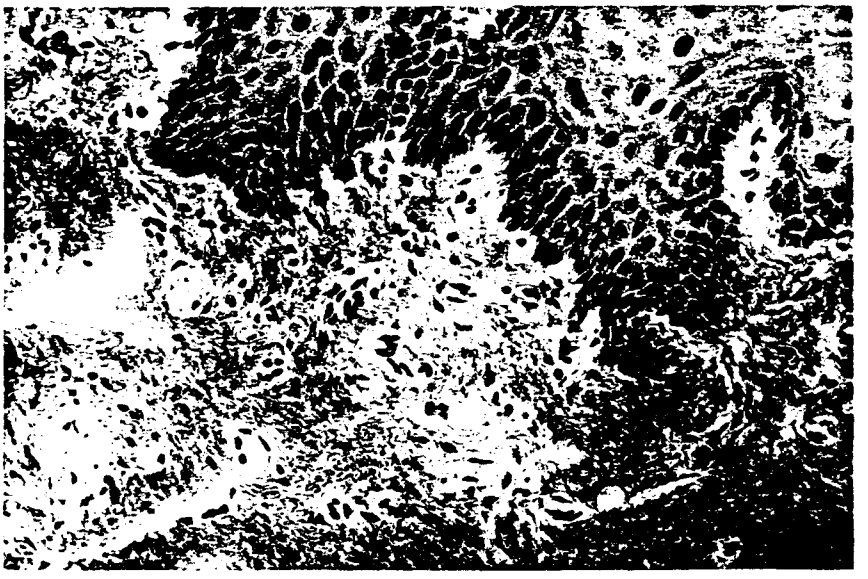

Fig. 4. Light-microscopic appearance of the test canal in a rat exposed to snuff for 13 months. Note the prominent fibrosis in the connective tissue and the mild inflammatory reaction (H&E, ×400).

The rats killed 4 months after termination of the snuff exposure, exhibited only slightly hyperplastic epithelium of the gingival sulcus (70%), with little or no keratinisation. The epithelial atrophy was less (30%) and only occasional ulcerations were seen.

The light microscopic appearance of the squamous epithelium of the buccal mucosa and the gingival sulcus of the control animals did not show any noteworthy pathological changes.

Discussion

All rats exposed to snuff for 13 months and then killed immediately exhibited lesions of the lip and oral cavity which were compatible with those described earlier (9, 10). The results of this study show that the described changes in the test canal after 13 months of snuff exposure followed by a snuff-free interval of 1–4 months are reversible to a certain extent. However, the squamous epithelium of the lips was atrophic and a few ulcerations were also seen after a snuff-free interval of 1–4 months. The subepithelial connective tissue exhibited extensive fibrosis.

The epithelium of the gingival sulcus of the lower incisors (crevicular epithelium) appeared to be more sensitive to snuff exposure than that of the tongue and buccal mucosa. This is probably due to the short distance between the test canal and the incisors. Furthermore, it seems likely that snuff is more or less constantly retained in the gingival sulcus, resulting in a longer exposure time in comparison with the buccal mucosa and tongue.

The gingival sulcus is covered with a thin unkeratinised epithelium, which may be more sensitive to snuff than the other locations, and this might explain the more pronounced microscopical changes found here.

Based on its content of tobacco specific nitrosamines – TSNA-snuff must be regarded as a carcinogenic agent (2). However, at the level present in snuff, especially in combination with a relatively short exposure time, the TSNA may predominantly act as promotors, resulting in proliferative lesions which to a large extent healed within 4 months after cessation of the snuff exposure.

We have previously reported on three snuff-induced intraoral squamous cell carcinomas using the same experimental model (11).

One tumour was detected after 9 months of snuff exposure and the other tumours were seen after 18 months of exposure to snuff and exposure to infectious Herpes simplex virus Type 1 (HSV-1, 12). All 3 tumours were located in close contact with the alveolar process. These tumours most likely originated from the gingival sulcus epithelium and not from the squamous epithelium of the test canal in the lip. This indicates that the squamous epithelium of the lip is rather resistant to exposure to snuff and chemicals in snuff such as the TSNA. The gingival sulcus area has also earlier been reported to be sensitive to effects of carcinogenic substances such as 4-nitroquinoline N-oxide, (4-NQO, 13).

It has been reported that squamous cell carcinoma may develop in the rat palate, even after as few as 3–6 applications of 4-NQO, if the latency time is sufficiently long (14). This suggests that the snuff-free interval before terminating this experiment may have been too short to result in tumour induction, or rather that snuff alone is a promotor or weak carcinogen requiring a much longer exposure time for tumour induction.

Although the lesions found in the test canal were found to be reversible to a certain extent, the canal serves as an excellent reservoir for the snuff. From this reservoir, snuff and products of snuff spill into the oral cavity for many hours. These substances may af-

Fig. 5. Light-microscopic appearance of the test canal in a rat exposed to snuff for 13 months followed by a snuff-free interval of 4 months. Note the rather atrophic squamous epithelium in comparison with rats exposed to snuff for 13 months (Fig. 4, H&E, ×400).

Fig. 6. Light-microscopic appearance of the epithelium lining the gingival sulcus in a rat exposed to snuff for 13 months. Note the moderate epithelial hyperplasia, increased keratinisation and subepithelial inflammation (H&E, ×250).

fect different organs throughout the body since they diffuse into the bloodstream and are excreted into the saliva and urine (2). The TSNA and other related substances in snuff may also interact with other substances such as ethanol (15) or HSV-1 as shown earlier (16). Further studies are under way to investigate these associations.

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