Harm and harm reduction in smokeless tobacco users

An *in vitro* and clinical study

Akademisk avhandling

som för avläggande av odontologie doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet
kommer att offentligen försvaras i föreläsningssal 3,
Medicinaregatan 12E, Göteborg, fredagen den 19 november 2010 kl 13.00

av

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This thesis is based on the following original publications, which will be referred to by their Roman numerals.

I. **Bengt Hasséus, Mats Wallström, Bengt-G. Österdahl, Jan-M. Hirsch, Mats Jontell.**
Immunotoxic effects of smokeless tobacco on the accessory cell function of rat oral epithelium.
Eur J Oral Sci. 1997 Feb;105(1):45-51.

II. **Mats Wallström, Lars Sand, Fredrik Nilsson, Jan-M Hirsch**.
The long-term effect of nicotine on the oral mucosa.
Addiction. 1999 Mar;94(3):417-23.

III **Mats Wallström, Gunilla Bolinder, Bengt Hasséus, Jan-M Hirsch**
A cessation program for snuff dippers with long-term and extensive exposure to Swedish moist snuff. A 1-year follow-up study.
Acta Odontol Scand. 2010 Sep10. [Epub ahead of print]

IV. **Mats Wallström, Magnus Kjelsberg, Anne Christine Johannessen, Jan-M Hirsch.**
The reversibility of the snuff-induced lesion – a clinical and histomorphological study.
(Submitted for publication September 2010.)

Göteborg 2010

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Harm and harm reduction in smokeless tobacco users. An *in vitro* and clinical study.

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Abstract:

Background: This thesis describes the effects of smokeless tobacco (ST) and its derivatives on the oral mucosa; the development of, and results of applying a cessation program for snuff users with long and extensive exposure; and the persistent clinical and histomorphological changes after cessation.
Methods: Three of the studies were open prospective non-randomized clinical intervention trials using nicotine replacement therapy (NRT) of either 4 mg nicotine chewing gum or 2 mg nicotine lozenges. Clinical examinations, biopsies, and histomorphological analyses were performed. In the fourth study *in vitro* assays were performed to investigate the effect of snuff extract, alkaloids, and selected tobacco-specific nitrosamines (TSNA) on the accessory function of rat oral epithelium cells and T cells.
Results: Of 280 participants motivated to discontinue snuff use, 50 were selected to treatment. After 3, 6, and 12 months, 58%, 46%, and 30% respectively were tobacco abstinent. Compliance was confirmed by measuring cotinine and carbon monoxide (CO) levels. Four subjects were still on NRT after 12 months, but tobacco-free since baseline. Twenty subjects abstinent after 6 months had a second biopsy from the site of snuff application. Of these, 40% showed remaining clinical lesions, the most significant of which were seen in the 75% of subjects still using NRT. The histomorphological picture was dominated by reductions in epithelial thickness, keratinization, and inflammatory response after tobacco cessation, although 30% of subjects showed increased epithelial thickness and 35% had increased or constant inflammatory reaction. A shift from ortho- to parakeratinization was noted in 80% of lesions. Of 30 individuals who used lozenges for 6 months, 8 presenting lesions had a significantly higher nicotine exposure ($p<0.05$) than those without lesions. All lesions appeared between 1 and 6 weeks after treatment began. After 3 months of NRT, all lesions had resolved but one, which was healed at the 6-month control. In the mitogen (concanavallin A) driven *in vitro* model using rat oral epithelium cells with accessory Langerhans cells (LC), T cells incubated with various concentrations of extract of Swedish moist snuff (SS) showed a significant inhibition of cell proliferation at 12.5% ($p<0.05$), and a concentration of 4% reduced T cell proliferation by 50%. Alkaloids and TSNAs in concentrations similar to those in SS had no significant effect on cell proliferation. No mitogenic capacity was detected in the SS extract, alkaloids, or TSNA, although *N′*-nitrosonornicotine (NNN) showed a tendency to be stimulatory in an *in vitro* assay with T-cells and rat oral epithelial cells.
Conclusion: Snuff cessation with NRT is a promising way to achieve a tobacco-free state. Compliance to treatment was high regardless of outcome, although almost all subjects gained weight, which correlated with a significant increase both in diastolic blood pressure in the success group and in total cholesterol values. Tissue samples from those with extensive exposure to snuff who were still using NRT on a daily basis 6 months after cessation were neither clinically nor histomorphologically completely normal. SS extract can evoke an immunosuppressive effect on T-cell proliferation using cells from oral epithelium as accessory cells. This effect was more pronounced when the complete SS extract was employed compared to when single components were used. These findings may indicate a local immunosuppressive effect of ST on the oral mucosa. Daily repeated sublingual exposure to nicotine for 3 months appears to be a safe form of administration with mild and transient effects in individuals devoid of clinical lesions.
Keywords: Cessation, nicotine replacement, oral mucosa, smokeless tobacco, snuff, reversibility, immunotoxicity, Langerhans cells, rat oral epithelium, nicotine.

ISBN: 978-91-628-8202-0