Histologic effects of smokeless tobacco and alcohol on the pouch mucosa and organs of the Syrian hamster

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This study's intent was to measure the histologic effects of smokeless tobacco and alcohol on the buccal pouch mucosa and internal organs of male Syrian hamsters. Eighty hamsters were divided into four groups: tobacco only, alcohol only, tobacco and alcohol, and negative control. 200 mg of smokeless tobacco were placed in each pouch of the tobacco groups five times a week. In the alcohol groups, 2 ml of 15% ethyl alcohol were placed in each pouch five times a week. The negative control group had mechanical stimulation of the right pouch to simulate the placement of the tobacco. After 26 wk the animals were sacrificed with pouches and abdominal organs removed. Alterations were observed in the abdominal organs, but not of statistical significance. However, significant acanthosis of the pouch epithelium was noted in the tobacco and tobacco and alcohol groups. This study reaffirms the lack of carcinogenic potential of smokeless tobacco upon the hamster pouch mucosa and internal organs.

Key words: acanthosis; alcohol; carcinoma; dysplasia; epithelial; papilloma; smokeless tobacco; Syrian hamsters

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Smokeless tobacco use has increased dramatically in the United States in recent years, especially among young men (1, 2). Also, chronic use of alcohol products has long been a dilemma for society (3). For individuals who partake of these substances, concurrent use is common (4). Regarding this relationship, extensive epidemiologic evidence has been accrued indicating the synergistic action of chronic alcohol consumption and tobacco use in the etiology of cancer of the oropharynx, larynx and esophagus (5–8).

Although numerous powerful carcinogenic compounds have been isolated from both smokeless tobacco and alcohol products (9, 10), experimental evidence of the carcinogenic potential of these whole agents on the pouch mucosa of the Syrian hamster has been limited (11, 12). In addition, few alterations have been observed in the gastrointestinal tract, liver and kidneys of animals exposed to these products (13, 14).

With these factors in mind, this study was designed to determine the histologic effects of smokeless tobacco and alcohol upon the pouch mucosa and internal organs of the male Syrian hamster.

Material and methods

Eighty male Syrian hamsters (Harlan Sprague Dawley, Indianapolis, IN) 8 months of age were divided equally into four treatment groups of 20 and balanced on the basis of body weight. Each group was maintained on a diet of ground Purina rodent chow and deionized water ad libitum. Group 1 animals were administered 200 mg moist/fine cut smokeless tobacco (pH = 7.7–8.1) (Skoal, U.S. Tobacco Co., Nashville, TN) into each buccal pouch via a 1 ml syringe once daily five times per week. Group 2 animals received 2 ml of 15% ethyl alcohol placed topically by a needle-less syringe in each buccal pouch once daily five times per week. Group 3 hamsters received 200 mg of Skoal in each pouch followed by the deposition of 2 ml of 15% ethyl alcohol once daily five times a week. Group 4 hamsters received mechanical manipulation by injecting air into the right pouch with a needle-less syringe to simulate tobacco placement. The left pouch remained untouched.

During the study the animals were examined weekly for gross abnormalities, such as tumors or surface color and texture alterations. At the end of 26 wk the animals were sacrificed by carbon dioxide inhalation. At that time the animals were assigned randomized code numbers for blind analysis. All pouches, livers, intestines, kidneys and adrenals were removed and placed in 10% neutral buffered formalin for 48 h. All pouches and organs were multisected at 2 mm intervals, taken through standard dehydration procedures and paraffin embedded. From each block 5 μm sections were prepared and stained with hematoxylin-eosin and coverslipped.

The tissue was then examined for epithelial and connective tissue alterations

Table 1. Pouch histopathology

Histopathology	Group ($n=20$) T	A	TA	C
Fibrosis	0	1	0	0
Inflammation	1	2	2	4
Atrophy	3	0	3	7
Acanthosis	14*	9	12**	4
Keratosis	19	18	20	20
Dysplasia (Grade 1)	3	4	4	4

T – tobacco only, A – alcohol only, TA – tobacco and alcohol, C – mechanical simulation only. * $P<0.005$, ** $P<0.025$.

Fig. 1. Normal buccal pouch histology. H&E, ×350.

via the light microscope. For the buccal pouches, the connective tissue was monitored for the presence/absence of inflammation and/or fibrosis. The epithelium was inspected for increased/decreased thickness, presence/absence of keratosis, presence/absence of ulceration and for alterations in morphology and cytology. If no alterations were observed, the sample was classified as normal (Grade 0). Dysplastic alterations were divided into four categories: mild (Grade 1), moderate (Grade 2), severe (Grade 3) and carcinoma-in-situ (Grade 4), each graded by thickness of involvement. Squamous cell carcinoma (Grade 5) was designated if connective tissue invasion was noted. These same parameters were utilized for the internal organs with additional observations noted were applicable.

Significance of variation of histologic results between individual groups was measured utilizing the chi-square test with Yates' correction due to the small sample numbers. P values of ≤ 0.05 were considered to be statistically significant.

Fig. 2. Increased epithelial thickness seen in Group 1 hamster. Tobacco particles can be seen at top (arrow). H&E, ×350.

Results

Gross alterations

No gross changes were noted during the course of the study.

Pouch histopathology

Histologic study of the pouch mucosa resulted in the findings presented in Table 1. The histologic findings of the right and left pouches of the control group were identical; therefore, these were considered together. Keratosis was noted in all groups and was indistinguishable between experimental groups. The only statistically significant histologic finding was the presence of increased epithelial thickness in more animals in groups 1 and 3 when compared with the negative control group ($P<0.005$ and $P<0.025$, respectively) (Figs. 1 and 2). Dysplastic features (Grade 1) were noted focally in all groups without significant variation.

Organ histopathology

Numerous alterations were observed in the organs of all groups (Table 2). Within the liver, all groups exhibited focal inflammation and fatty change. An interesting finding was the presence of biliary microcysts (Fig. 3). However, the variation between groups was not statistically significant. Chronic glomerulosclerosis was noted only in groups 1 and 4 (Fig. 4), but again was not significant when statistical analysis was employed. The intestines did not exhibit major al-

Table 2. Organ histopathology

	Group ($n=20$)			
	T	A	TA	C
Liver				
Inflammation	7	9	8	9
Hemangioma	0	1	0	0
Fatty change	6	7	6	5
Microcysts	2	2	1	4
Kidney				
CGS	3	0	0	2
Intestines				
Inflammation	1	2	0	0
Adrenal				
Adenoma	2	1	1	1
Mesentery				
Necrosis	1	0	1	2
Stomach				
Inflammation	9	12	10	13
Ulceration	6	4	6	3
Hyperkeratosis	7	7	9	13
Papilloma	2	3	4	0
Dysplasia (Grade 1)	4	3	4	5

T – tobacco only, A – alcohol only, TA – tobacco and alcohol, C – negative control, CGS – chronic glomerulosclerosis.

Fig. 3. Biliary microcysts of the liver in Group 2 hamster. H&E, ×100.

terations. Adenomas of the adrenal gland were noted with equal frequency in all groups.

Changes were found in the forestomach of all groups. Focal ulceration and inflammation were observed in each group without significant variation. Papillomas were observed in all three treatment groups, but not in the control group (Fig. 5). However, the numbers present were not significantly different. Focal dysplastic alterations (Grade 1) were found in all four groups equally. Another finding which was noted in groups 1, 3 and 4 was the presence of circumscribed necrosis within the mesentery unattached to the gastrointestinal tract.

Discussion

The only statistically significant histologic feature produced by this study was the presence of increased epithelial thickness in the groups exposed to tobacco alone as well as tobacco and alcohol. This is consistent with findings observed in human smokeless tobacco users and previous experimental studies (15–17). A follow-up study has indicated that these changes appear to be reversible phenomena once usage of tobacco is terminated (18).

No statistical difference was observed in the rate of dysplastic change or was the formation of carcinoma noted in the pouch mucosa. This may have resulted from the relatively short term of the study (26 wk), from too small of dosage of the alcohol and tobacco or from insufficient frequency of exposure. In addition, the potential for carinogenesis of Skoal may not be sufficient to induce neoplastic change in this particular animal model. It has been shown that smokeless tobacco and alcohol require relatively long term exposure for carcinogenic transformation to occur and that frequency and dosage are integral parts for this transformation (19, 20). It should be noted, however, that tobacco of a different character (dry snuff) was used in one study and thus may behave differently than the tobacco product utilized for this current study (19). Also, only one carcinoma was produced in the latter study (20).

This study demonstrated several internal pathological findings, although none statistically significant. Microcysts of the biliary ducts of the liver were seen in all groups, a condition previously reported to occur spontaneously in hamsters (21) and humans (22). Papillomas also have been reported to occur spontaneously in the forestomach of Syrian hamsters (23). The rate of occurrence in this study, 10–20%, is similar to what has been reported in tobacco extract studies (24).

Another feature in the abdomen which defies explanation was the presence of foci of necrosis in the mesentery of animals in the tobacco groups and in the negative control group. To our knowledge, this finding has not been previously described in the literature.

This study demonstrated the ability of smokeless tobacco to induce increased epithelial thickness of the pouch mucosa, which has been shown to be reversible when usage of tobacco ceases. Otherwise, the short-term effects of smokeless tobacco and alcohol on the pouch mucosa and internal organs of the male Syrian hamster are relatively few. This study confirms the inability of smokeless tobacco and alcohol to produce neoplastic or preneoplastic alterations in the hamster pouch mucosa. This indicates that the hamster pouch is an inadequate locale for carcinogenesis of tobacco products and that this particular tobacco product, Skoal, exhibits no

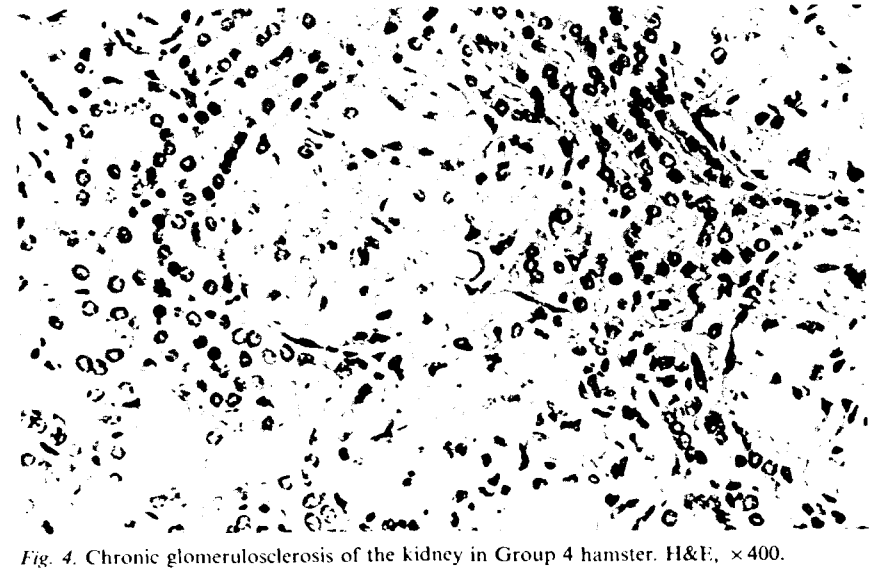

Fig. 4. Chronic glomerulosclerosis of the kidney in Group 4 hamster. H&E, ×400.

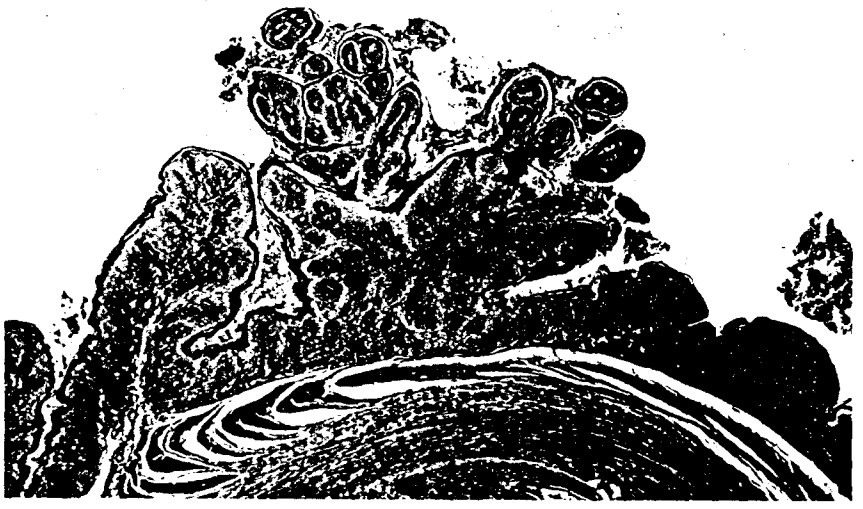

Fig. 5. Forestomach papilloma in Group 3 hamster. H&E, ×45.

carcinogenic potential in this animal model.

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