Scanning Electron Microscopy of the Tongue, Pharynx, and Larynx of Rats Exposed to Cigarette Smoke

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Summary: Objective. To examine, by using scanning electron microscopy, the surface of the tongue, pharynx, and larynx of animals exposed to tobacco.
Study Design. Experimental study.
Methods. Twenty rats were allocated to two groups: group I, control group: 10 rats not exposed to any inhaling pollutant and group II, tobacco group: 10 rats exposed to smoke from 10 cigarettes twice a day for 260 days. Animals of both groups had no restriction of food or water. After those 260 days, their aerodigestive segment was removed, and fragments of their tongue, hypopharynx, and right vocal fold were immersed in 2.5% glutaraldehyde and prepared for scanning electron microscopy.
Results. The filiform tongue papillae of the tobacco group were irregularly displayed, flattened, and adhered to each other. The hypopharynx mucosa was highly irregular, thickened, rough and had increased superficial peeling. The mucosa of the vocal folds had deep furrows surrounding the cells. These alterations were not identified for the control group.
Conclusion. Several changes were recorded for the tongue, pharynx, and larynx of tobacco group animals, confirming the harmful effects of smoking to the respiratory and digestive epithelium.
Key Words: Tobacco–Smoking–Scanning electron microscopy–Rats–Tongue–Pharynx–Larynx.

INTRODUCTION

The oral cavity, the larynx, and the pharynx are responsible for 30–40%, 25%, and 15%, respectively, of head and neck carcinomas, and smoking has been reported by 95% of these patients.[1–4] Cigarette smoke contains a large number of harmful chemicals, especially carbon monoxide, nicotine, and tar. Cellular absorption of tobacco's noxious agents may lead to DNA mutations, triggering the carcinogenesis process.[5]

Experimental studies have used different methods to reproduce lesions in the airways of animals by exposing the latter to cigarette smoke. Mucosal responses to these harmful agents include hyperplasia, hyperkeratosis, dysplasias, and metaplasia of the epithelium.[6–12] Some of these lesions were already shown by one of our studies exposing rats to cigarette smoke for 60 and 260 days[12,13] and confirmed by different animal studies.[8–11] Those authors, however, did not include electron microscopic analysis, which may add important information.

The aim of this study was to examine, by using scanning electron microscopy, the surface of the tongue, pharynx, and larynx of animals exposed to cigarette smoke.

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MATERIAL AND METHODS

This project was approved by the Animal Experimentation Ethics Committee of Botucatu Medical School, UNESP-Univ Estadual Paulista (698/2008). Twenty adult Wistar rats weighed approximately 180–200 g were kept in individual cages under acclimatized environment (temperature of 23°C ± 2°C and humidity of 60% ± 5%) and allocated to two study groups:

Group I, control: 10 rats received water and animal food *ad libitum* during 260 days;
Group II, tobacco: 10 rats were exposed to smoke from 10 cigarettes twice a day during 260 days, without food restriction.

Exposure to cigarette smoke was carried out by transferring group II animals (n = 10) to a chamber connected to a "smoking device." Puffs of smoke were vacuum aspirated from cigarettes and introduced into the chamber during 30 minutes twice a day for 260 days; then, the chamber was opened to allow the smoke out.[8] The used cigarettes had the following composition: 1.1 mg nicotine, 14 mg tar, and 15 mg carbon monoxide.

The animals were killed at 260 days after the beginning of the experiment by means of intraperitoneal pentobarbital sodium (50 mg/kg). The aerodigestive segment containing the tongue, the pharynx, the larynx, and the first tracheal rings were removed and dissected (Figure 1). Each specimen was macroscopically examined throughout its extension and, in the absence of lesions, standardized biopsies were performed for the following sites: base of tongue, right lateral hypopharynx, and right vocal fold. The obtained 0.6–0.8 cm fragments were immediately immersed in 2.5% glutaraldehyde solution and subjected to processing.

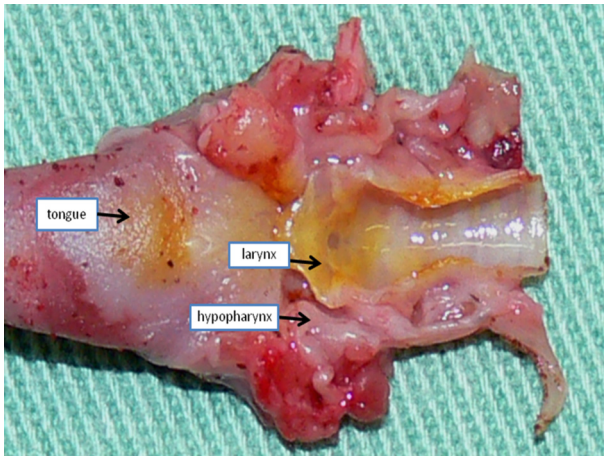

FIGURE 1. Removed airway segment and biopsied sites.

Preparation for scanning electron microscopy

After fixation using 2.5% glutaraldehyde for 48 hours, fragments were washed in phosphate buffer, 0.1 M, pH 7.3, fixed with 1% osmium tetroxide solution, washed in phosphate buffer, dehydrated in alcohol solutions at increasing concentrations, 75–100%, and dried in a critical point device (Balzers CPD-020; Union, Liechtenstein) using liquid carbon dioxide. The specimens were mounted on a metal base using silver glue and covered with gold in a Balzers MED-010 device (Liechtenstein). Then, each quadrant was blindly examined by an experienced pathologist under a scanning electron microscope at increasing magnifications (model Quanta 200 FEG; FEI Company, EUA), under 15 KV tension.

RESULTS

Tongue

Control animals had their tongue surface covered with regularly displayed filiform tongue papillae (Figure 2). However, all smoke-exposed animals had irregularly displayed papillae that were frequently flattened and adhered to each other (Figures 3 and 4). Accumulation of food debris and mucus on the papillae were also found.

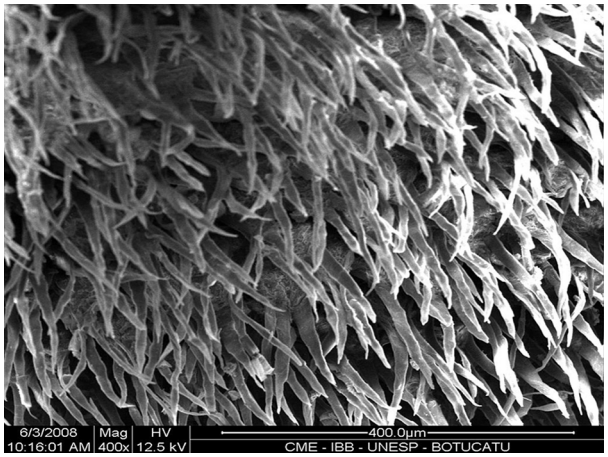

FIGURE 2. Tongue surface of a group I animal (control). Uniform arrangement of filiform papillae. Scanning electron microscope, ×400.

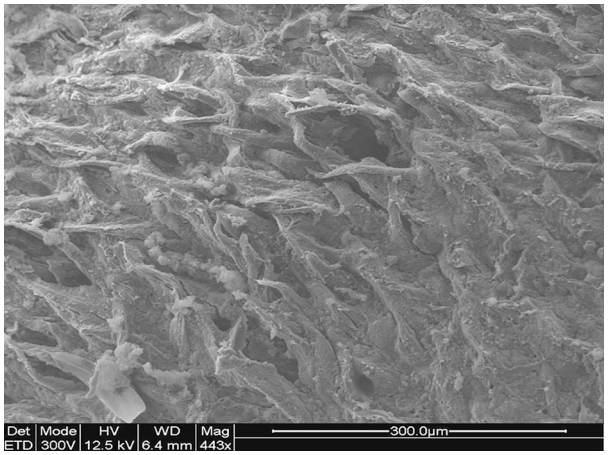

FIGURE 3. Tongue surface of a group II animal (tobacco). Dysmorphic and asymmetrical aspect of the filiform papillae. Scanning electron microscope, ×443.

Hypopharynx

For control animals, the hypopharynx fragments examined under a scanning electron microscope showed highly folded surface, which allows this organ to distend during feeding, and some cells were detaching from the surface (Figure 5). For smoke-exposed animals, the hypopharynx surface was greatly irregular, thickened, rough and had increased superficial peeling (Figures 6 and 7). The covering mucosa had "brilliant, impermeable, and smooth" aspect.

Larynx

For control animals, the surface of vocal folds was less folded and had some detaching cells (Figure 8). On the other hand, smoke-exposed animals had deep furrows surrounding the cells on the surface of vocal folds, similarly to microclefts (Figure 9).

DISCUSSION

Smoking is responsible for the development of several lesions in the airways including inflammation, acanthosis, hyperkeratosis, dysplasias, leukoplakias, erythroplakias, and carcinoma. These

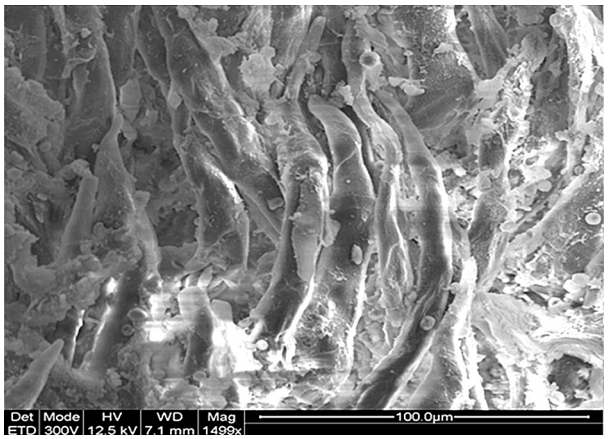

FIGURE 4. Tongue surface of a group II animal (tobacco). Flattened filiform papillae adhered to each other. Food debris and mucus deposited on the papillae. Scanning electron microscope, ×1499.