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BASIC RESEARCH

Does cigarette smoke exposure lead to histopathological alterations in the olfactory epithelium? An electron microscopic study on a rat model

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ABSTRACT

Objective: This study was conducted to examine the influence of smoke exposure of variable duration on the ultrastructure of and histopathologic and morphologic alterations in the olfactory epithelium.
Methods: A total of 24 Wistar albino rats were randomly assigned to three groups and fed a standard rat chow and tap water. Experimental rats in groups I and II were exposed to cigarette smoke in a glass cabin over a period of 2 months for 5 or 15 min, respectively, four times daily; control rats (group III) were not exposed to cigarette smoke. After dissection, all tissue specimens were processed using routine procedures for TEM.
Results: Groups I and II exhibited the presence of intraepithelial inflammatory cells and especially deep invaginations in the nuclear membrane of supporting cells. Extended intercellular spaces, cytoplasmic protrusions on the apical surface of supporting cells, atrophy of microvilli and olfactory neuron cilia as well as numerous electron-dense granular structures and lysosome-like structures were observed to an increasing degree from group I to group II. Particularly in group II, both supporting cells and olfactory neurons exhibited a cytoplasmic edema, mitochondrial degeneration, and numerous vacuolar structures, as well as apoptotic and minimal necrotic changes. In this group, hyperplasia of basal cells was also observed.
Conclusion: Our electron microscopic findings show that cigarette smoke leads to toxic degenerative changes in the rat olfactory mucosa.

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Electron microscopy; olfactory mucosa; passive smoking

Introduction

The olfactory epithelium, a pseudostratified, ciliated epithelium, is superiorly located within nasal passages. The epithelium is composed of the following cell types: olfactory cells (bipolar neurons); smell receptors; supporting (sustentacular) cells; tall columnar cells, which provide mechanical and metabolic support to olfactory cells and have numerous long microvilli extending from their apical surface; basal cells; stem cells, from which new olfactory cells and supporting cells differentiate; and brush or microvillar (M) cells, functioning in general sensation rather than olfaction.[1,2] Normally, there are no goblet cells.

The apical pole of each olfactory neuron is thickened as a knob-like modified dendrite enlarged into the olfactory vesicle. A number of cilia extend radially from the olfactory vesicle in a plane parallel to the epithelial surface. They are thought to contain receptors for smelling. More proximally, the cell body widens to accommodate the nucleus and then narrows, in a fusiform fashion, to form the axon. The axonal terminal leaves the epithelial compartment by penetrating the basal lamina to form bundles of nonmyelinated nerve fascicles, the *fila olfactoria*.[1–3] It is known that certain inhalants, particularly cigarette smoke, may cause irritation and consequent toxic and neoplastic changes in the respiratory system.[4–10] As the exposure time increases, the tissue damage becomes more severe.[11,12]

Smoking is considered a widespread public health problem globally. In addition to other proven results, the deficit in the olfactory sense is six times more common in smokers than in nonsmokers.[13,14] The aim of this study was to examine the ultrastructure of olfactory epithelia in rats exposed to cigarette smoke for varying lengths of time and observe histopathological tissue alterations more accurately via transmission electron microscopy.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/iusp.

Materials and methods

In the present study, 24 adult Wistar albino rats, weighing 250–300 g, were obtained from the Experimental Research and Application Center of Istanbul Medipol University. All animals were handled according to the animal handling protocol approved by the institutional board (IMU-HADYEK). These animals were randomly divided into three groups and maintained under standardized conditions of light (12-h light/dark cycle) and temperature (22 ± 2°C), being fed a commercial rat diet and fresh tap water for 2 months. Experimental rats were exposed to cigarette smoke four times daily for 2 months in an automated glass cabin [Figure 1] for smoke exposure.[15] Control animals (group III) were treated in the same cabin but only exposed to fresh air. The experimental animals of groups I and II were exposed to cigarette smoke for 5 or 15 min, respectively. After 2 months, the animals were sacrificed by cardiac perfusion with 2.5% glutaraldehyde in 0.1 M phosphate buffer after being deeply anesthetized with ketamine. After the nasal tissues were removed from the animals, the olfactory mucosa was carefully dissected from the bone and placed in the same fixative. Following initial fixation, tissues were postfixed in 2% phosphate-buffered osmium tetroxide, dehydrated in acetone, and embedded in *Araldite CY 212*. Blocks of tissue were sectioned with glass knives on a *Nova LKB Bromma* ultramicrotome. Semithin sections were stained with toluidine blue for orientation. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a *Jeol 100 SX* electron microscope.[16]

Results

In the control group, olfactory cells (bipolar neurons) were characterized by a spindle-shaped form, apical olfactory vesicles, and an electron-lucent cytoplasm. The olfactory vesicles had many sensory cilia. Basal bodies of the cilia were observed within the lateral margins of the vesicle. On the epithelial surface, there were numerous transverse, longitudinal, and tangential profiles of cilia. The thick proximal portion of cilia had a typical "9 + 2" microtubule arrangement. Ciliary narrow regions, containing a few microtubules at the tip, were also observed. Some vesicles, which may have represented a smooth endoplasmic reticulum (SER) or exocytic or endocytic vesicles, were present in the apical portion of olfactory cells. These cells were linked by junctional complexes with adjacent supporting cells. Supporting cells had numerous long, branched microvilli extending from their apical surface. The microvilli were entangled with olfactory cilia and microvilli of neighboring cells on the epithelial surface. The cytoplasm of supporting cells was denser than that of adjacent olfactory neurons. Well-developed terminal webs were observed in the apical portion of supporting cells, separating the organelle-poor apical cytoplasm from a deeper region, where a number of mitochondria were present (Figure 2). Basal cells, small and polygonal or irregular in profile, were located on the basal lamina. Their cytoplasm, surrounding a usually indented heterochromatic nucleus, had prominent tonofilaments. Numerous cytoplasmic processes, extending from these cells, appeared to interdigitate with those of neighboring cells and to envelop the axonal

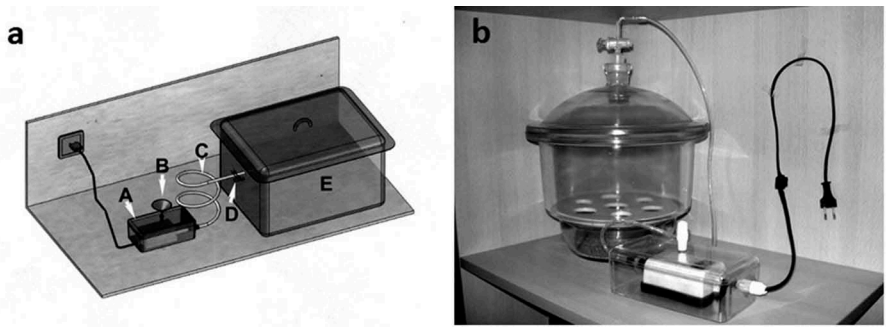

Figure 1. **a:** Shows technical drawing of the automated exposure apparatus of the experiment. **A.** Cabin forming negative pressure and maintains the lite of smoke. **B.** Cigarette placement handle **C.** Smoke stream **D.** On-off controller for stream **E.** Cabinet for experiment rats.

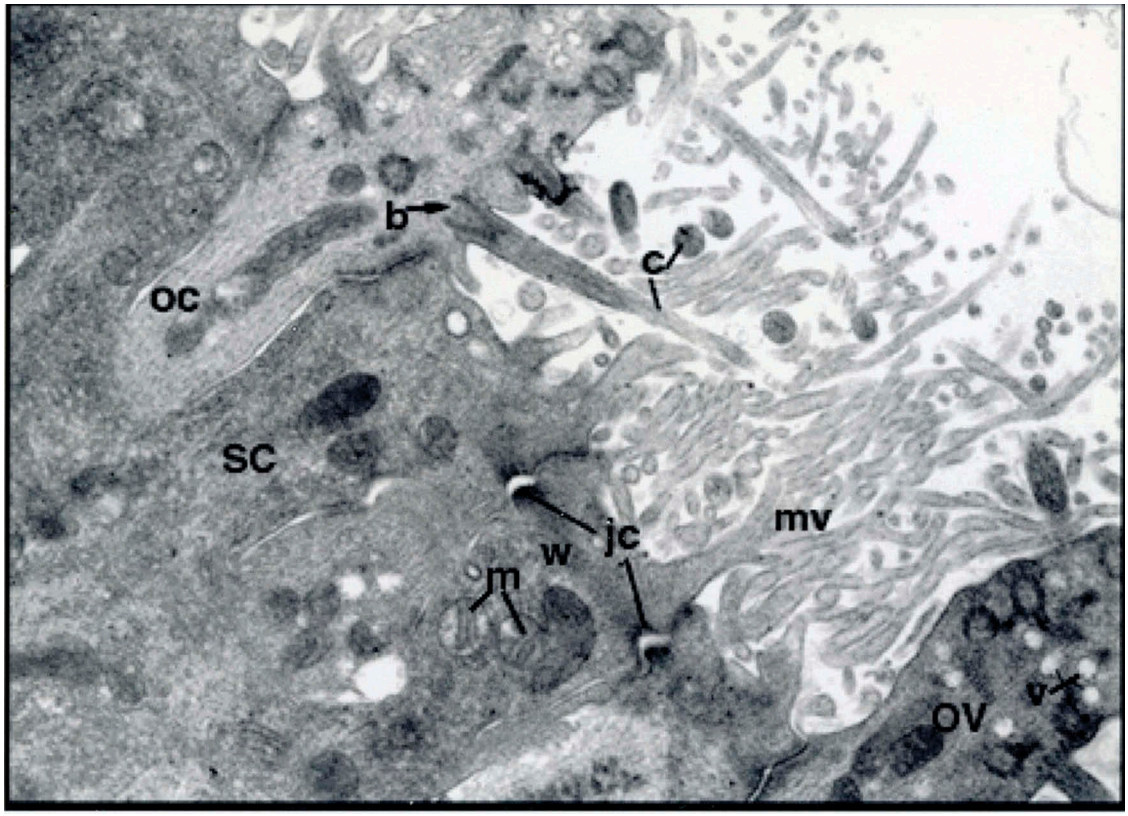

Figure 2. Electron micrographs from the olfactory epithelium of control rats.OC, olfactory cell; SC, supporting cell; OV, olfactory vesicle; c, cilia; mv, microvilli; b, basal body; v, vesicles; jc, junctional complexes; w, terminal web; m, mitochondria. Uranyl acetate and lead citrate Original Magnification (O.M.). x 800.

termini of olfactory neurons (Figure 3). In the basal portion of the olfactory epithelium, there were ducts entering the epithelium to deliver the secretion of Bowman's glands, situated in the lamina propria, to the olfactory surface. Duct cells, squamous in shape, had microvilli and cilia on their luminal surface, as well as cytoplasmic granules (Figure 4).

Prominent ultrastructural alterations were noted in olfactory epithelia of the rats exposed to cigarette smoke. In both experimental groups, degenerating cells and intraepithelial inflammatory cells such as lymphocytes and plasmocytes were observed. In olfactory epithelia from group I, electron-denser supporting cells with an invaginated nuclear membrane were encountered among normal ones (Figure 5). In group II, the supranuclear region in supporting cells contained large numbers of round or irregular inclusions filled with an electron-dense osmophilic material (Figure 6). In group III, supporting cells exhibited luminal ballooning or protrusions (Figures 8 and 9). In both supporting cells and olfactory neurons, large amounts of transverse or longitudinal SER profiles, mitochondrial degeneration and vacuolization, intracytoplasmic large vacuoles, and cytoplasmic edemas were present to an increasing degree from group I to group II (Figures 7 and 8). Group II tissues were also particularly notable for numerous apoptotic cells, characterized by shrinkage of the cytoplasm, condensation of the chromatin to the periphery of the nucleus, and expanded intercellular spaces (Figure 8). This group showed, in addition, a decrease in the thickness of the microvillous border, lying on the luminal surface (Figure 7), degeneration in olfactory vesicles, from which cilia emerge, and a reduction in the number of microvilli (Figure 6) compared with those in the control group. In both experimental groups, junctional complexes between olfactory neurons and supporting cells on the epithelial surface remained intact (Figure 7), and apart from this, many light basal cells with a euchromatic

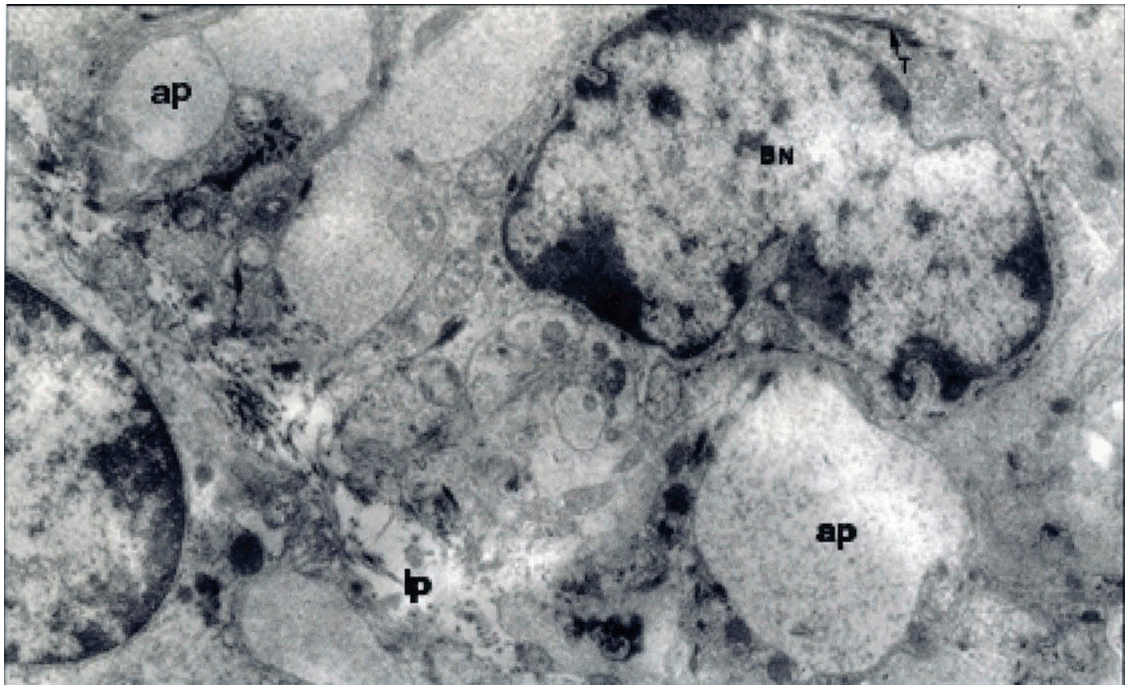

Figure 3. An electron micrograph from normal rat olfactory mucosa. BN, the nucleus of the basal cell; ap, axonal process of the olfactory neuron; lp, lamina propria; T, the bundles of tonofilaments. Uranyl acetate and lead citrate (O.M. x 6000).

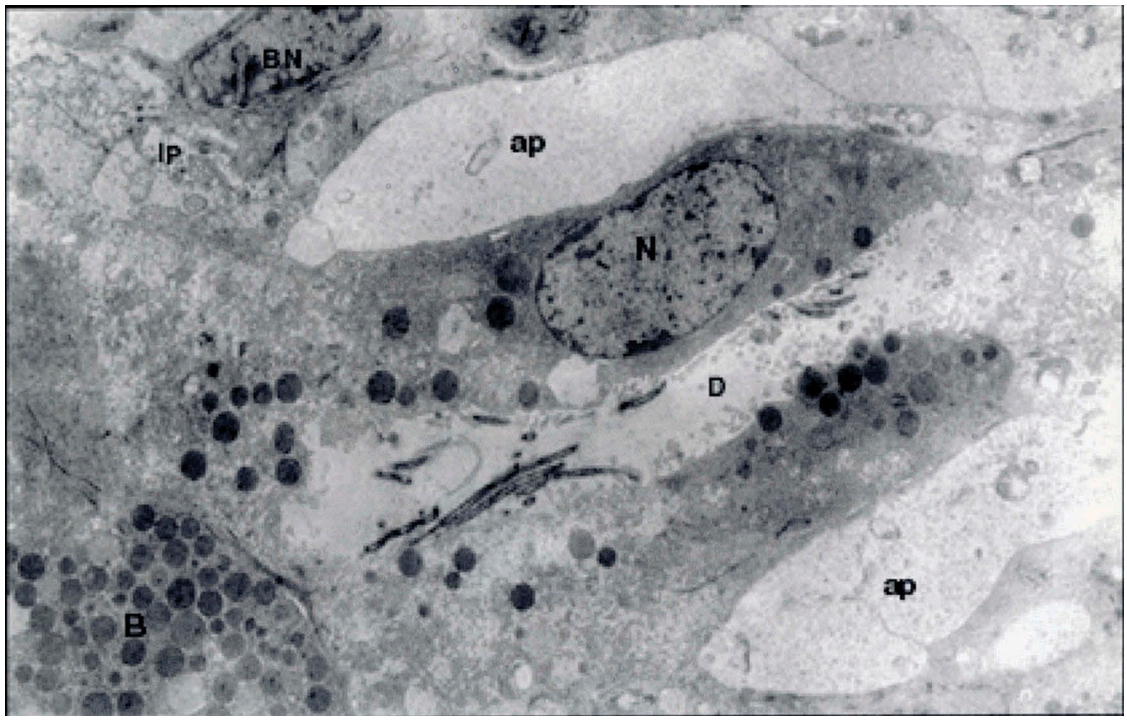

Figure 4. An electron micrograph from normal rat olfactory mucosa.D, the lumen of a duct entering the olfactory epithelium; N, the nucleus of a duct cell; BN, the nucleus of a basal cell; lp, lamina propria; ap, axonal process; B, a portion of Bowman's gland. Uranyl acetate and lead citrate-(O.M.).

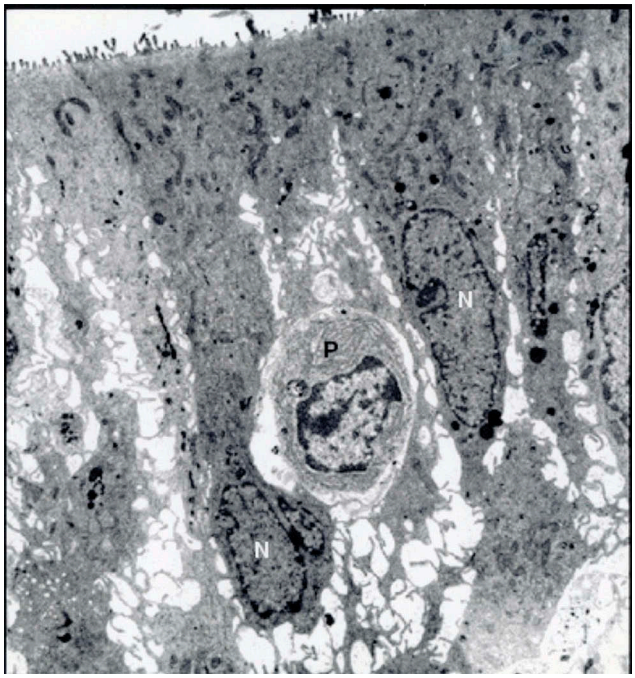

Figure 5. An electron micrograph of the olfactory epithelium from Group I. N, the nucleus of the supporting cell; P, an intraepithelial-plasmocyte. Uranyl acetate and lead citrate, O. M.x 3000.

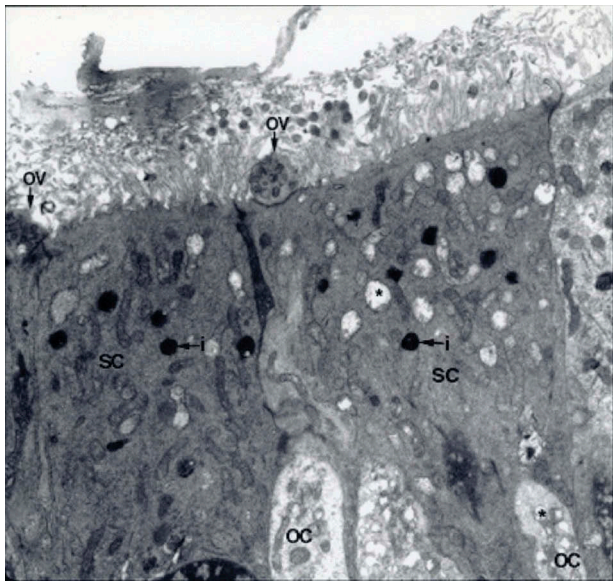

Figure 6. An electron micrograph of a portion of the olfactory epithelium from Group II.OC, olfactory cell; SC, supporting cell; OV, olfactory vesicle; i, electron-dense inclusions; *, mitochondrial swelling. Uranyl acetate and lead citrate, O.M. x 5000.

nucleus and an electron-lucent cytoplasm were encountered among more common dark basal cells (Figure 9).

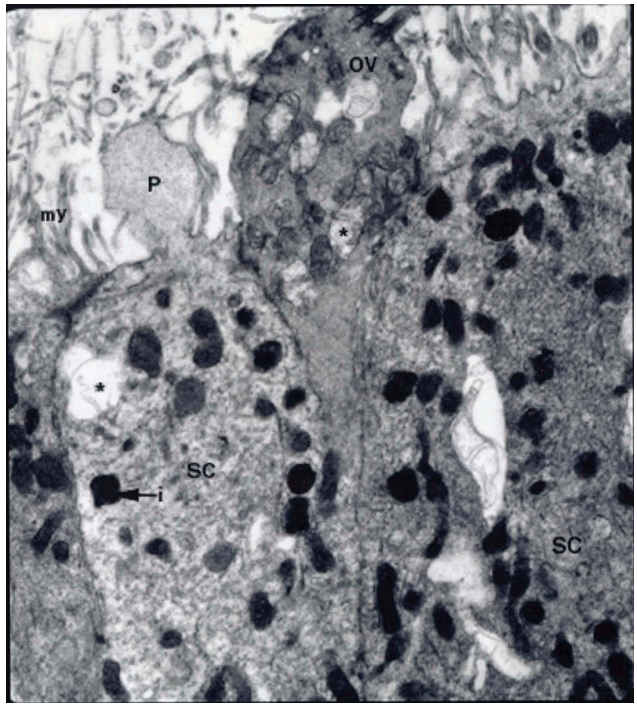

Figure 7. An electron micrograph of a portion of the olfactory epithelium from Group III.SC, supporting cell; OV, olfactory vesicle; i, electron-dense inclusions; *, mitochondrial swelling and degeneration; p, a protrusion of the supporting cell; mv, microvilli. There is a prominent cytoplasmic edema in the supporting cell on the left. Uranyl acetate and lead citrate, O. M. x 8000.

Discussion

The electron microscopic structure of the normal rat olfactory epithelium, observed in this study, was similar to that reported in the literature.[2,17,18] In olfactory epithelia of the experimental groups, swollen mitochondria, intracytoplasmic vacuolar structures, cytoplasmic edemas, and expanded intercellular spaces were noted. It is known that there are many toxic materials in cigarette smoke.[4,19] Upon toxic exposure, cell swelling has been reported to occur, owing to an impairment of the sodium pump mechanism in the cell membrane. In addition, it has been reported that mitochondria are important direct or indirect targets for virtually all types of injurious stimuli, including toxins, and mitochondrial swelling occurs under toxic conditions.[20] In our opinion, the vacuolar structures may have occurred owing to mitochondrial swelling and degeneration. Moreover, some researchers have reported that mitochondrial dysfunction, occurring under toxic conditions, causes an increase in cytosolic calcium,

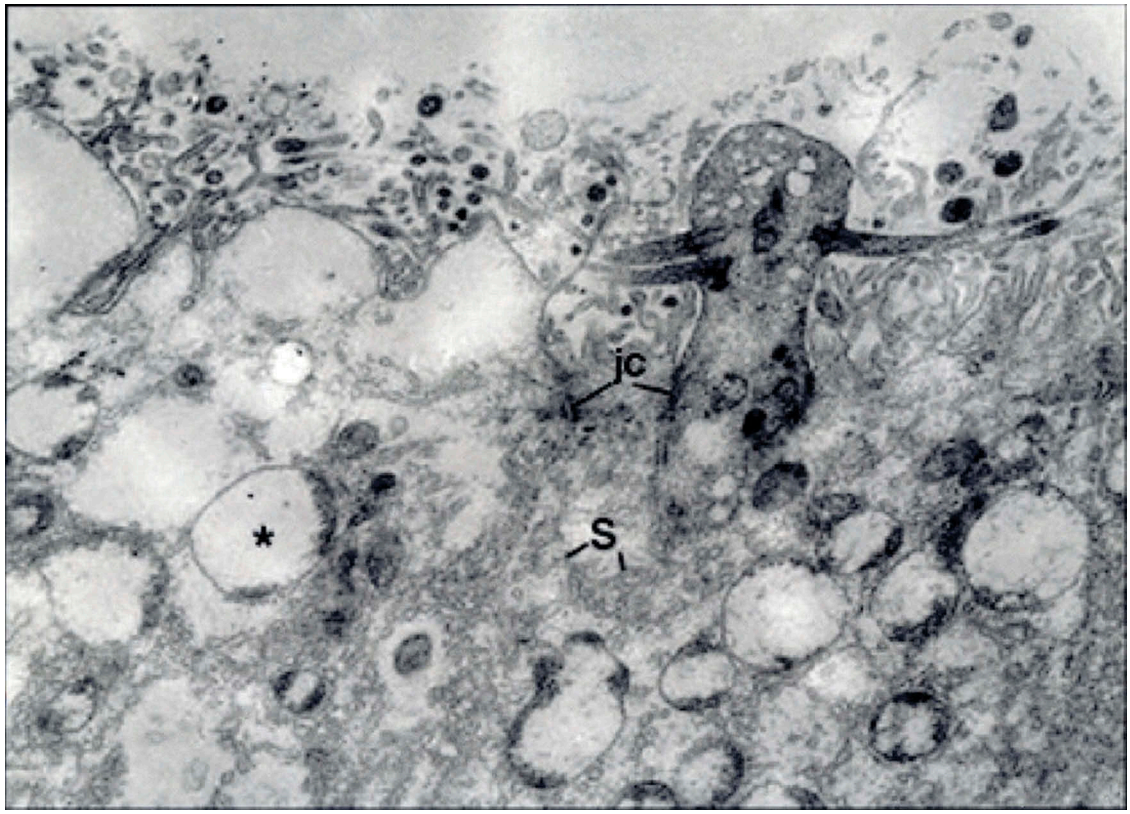

Figure 8. Electron micrographs of apical portions of the olfactory epithelia from Group III. There are a prominent cytoplasmic edema and the luminal ballooning in the supporting cells, and a decrease in the thickness of the microvillous border in comparison with that of the control group. *, vacuolar structures; S, SER-profiles; jc, junctional complexes. Uranyl acetate and lead citrate, O.M. x 8000.

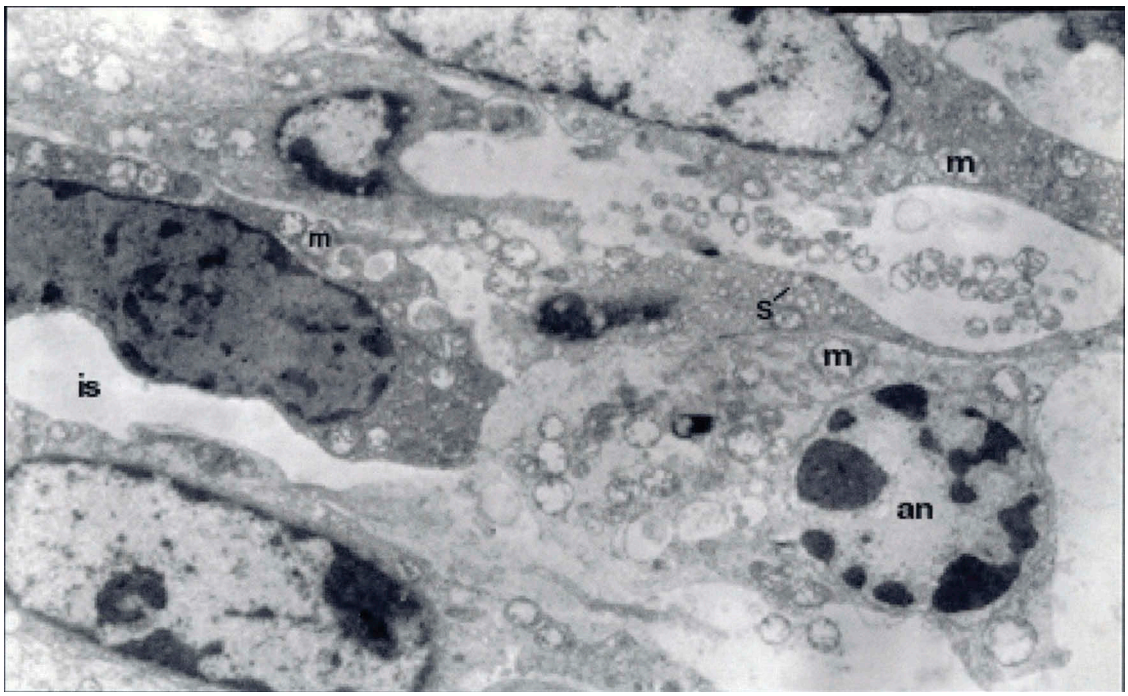

Figure 9. Electron micrographs of the olfactory epithelium from Group III.Many degenerated cell are seen. S, SER-profiles; an, apoptotic nucleus; is, expanded intercellular spaces; m, degenerated mitochondria. Uranyl acetate and lead citrate, O.M. x 4000.

which in turn results in damage to elements of the cytoskeleton.[20] It has been thought that widening of intercellular spaces and protrusion of the apical cytoplasm of supporting cells may be related to cytoskeletal damage. In supporting cells of the smoke-treated groups, a large amount of transverse or longitudinal SER profiles and abundant irregular inclusions, containing an osmophilic material, were present. It is known that SER has significant functions in detoxification processes.[2,17] Under toxic conditions, therefore, excessive development of SER is normal. In our opinion, the irregular, electron-dense inclusions may be secondary lysosomes, lipofuscin granules or aggregates of the olfactory pigment. These structures may reflect an increase in cellular digestion of organelles damaged after smoke exposure or of toxic materials in inhaled cigarette smoke. Apart from these, many mononuclear cells such as lymphocytes and plasmocytes were observed in olfactory epithelia of the experimental groups. Under toxic situations, the increase in inflammatory cells may be due to an immunological response to tissue damage.[20] Moreover, many apoptotic cells were observed in olfactory epithelia of the experimental groups. It has been reported that apoptosis, or programed cell death, involves the activation of a pathway that leads to a suicide of the cell via a characteristic process wherein the cell becomes more compact, blebbing occurs in the membranes, the chromatin becomes condensed, and DNA is fragmented.[21] Apoptosis can be triggered by a variety of stimuli, including toxins.[22–24] Particularly in group II, a decrease in the thickness of the microvillous surface, degeneration of olfactory vesicles, and a reduction in the number of microvilli were noted. Other authors have reported findings similar to ours in the olfactory epithelium treated with pyridine, nickel or sulfur dioxide.[25–27] Talhaut et al[19] have reported that tobacco smoke contains more than 5000 different kinds of toxic chemicals, including pyridine and nickel. In addition, in the experimental groups, many light basal cells with a euchromatic nucleus and a pale cytoplasm were observed among dark ones. It is known that basal cells are stem cells that differentiate to replace olfactory neurons and supporting cells lost during a normal turnover or injury, and basal cell hyperplasia follows the olfactory cell loss.[11,17,18,28] As basal cells begin to differentiate, their shapes become globose, and nuclei become euchromatic.[18,29] In our opinion, the light basal cells may be basal cells differentiating to replace damaged cells after smoke exposure.

In conclusion, we report that cigarette smoke causes serious tissue damage in the rat olfactory epithelium and that tissue injuries become more severe with an increasing exposure time.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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