

ORIGINAL RESEARCH

Rhinitis medicamentosa: Electron microscopic changes of human nasal mucosa

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OBJECTIVE: Prolonged application of nasal vasoconstrictors causes rhinitis medicamentosa (RM). Nasal obstruction is induced by rebound swelling when the decongestive effect has disappeared. The aim of this study was to demonstrate ultrastructural changes in RM.

STUDY DESIGN AND SETTING: Tissue samples of inferior turbinates from 22 patients with RM and 10 patients without rhinitis were taken during nasal surgery. Ultrathin sections were investigated by using a transmission electron microscope (TEM).

RESULTS: The TEM findings revealed severe epithelial damages such as loss of ciliated cells. In the subepithelial region, the vascular endothelium showed gaps and ruptures of basal lamina.

CONCLUSION: RM is a drug-induced damage of human nasal mucosa. Loss and destruction of ciliated epithelial cells are the morphologic correlation of the disturbed mucociliary clearance. In addition, vascular endothelium revealed ultrastructural changes. This could be caused by an increased vascular permeability with consecutive interstitial edema.

SIGNIFICANCE: This study demonstrated new morphological aspects of rhinitis medicamentosa.

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For many decades, topical vasoconstrictors, ie, local alpha-sympathomimetics, have been used for diagnostic purposes as well as short-term therapy of obstructive diseases of the nasal mucosa. Their decongestant effect is especially useful in cases of temporary turbinate hyperplasia. Topical intranasal decongestive drugs include two classes, both sympathomimetic amines (eg, phenylephrine) and imidazoles. Oxymetazoline and xylometazoline, the topically applied imidazoles

interact with the postsynaptic α_2 and α_1 receptors of the arterial resistance vessels, and cause a quick-acting vasoconstriction.^{1,2} The imidazoles appear more likely to cause rebound swelling and rhinitis medicamentosa than are the sympathomimetic amines because of their longer duration of pharmacologic effect on mucosal blood vessels.³

With repeated application and long-term use of these drugs, a tachyphylaxis with shorter efficacy intervals and increased application of the nasal spray develops, which has been known since the 1940s.⁴⁻⁶ The prolonged use of these nasal decongestants induces nasal obstruction. The nasal stuffiness is caused by rebound swelling, and the patient starts to use larger doses of these drugs more frequently. This vicious circle has to be broken with professional help.

Studies that concerned morphologic changes of nasal mucosa after application of nasal decongestants are rare and affect predominantly animal experiments⁷⁻¹⁰ or were carried out with a scanning electron microscope.¹¹ The aim of our investigations was to evaluate changes of human nasal mucosa, especially with the focus on epithelial cells and endothelial lining of mucosal vessels, after prolonged use of topical vasoconstrictors. A transmission electron microscope was used in the comparison with normal nasal mucosa.

MATERIALS AND METHODS

Sampling of nasal mucosa

Tissue samples of the anterior third of inferior turbinates were taken during nasal surgery from 22 patients, 10 fe-

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Presented at the Annual Meeting of the American Academy of Otolaryngology–Head and Neck Surgery Foundation, Sept 17-20, 2006, in Toronto.

Financial support was provided by the German Research Society (DFG, KN 501/1-2).

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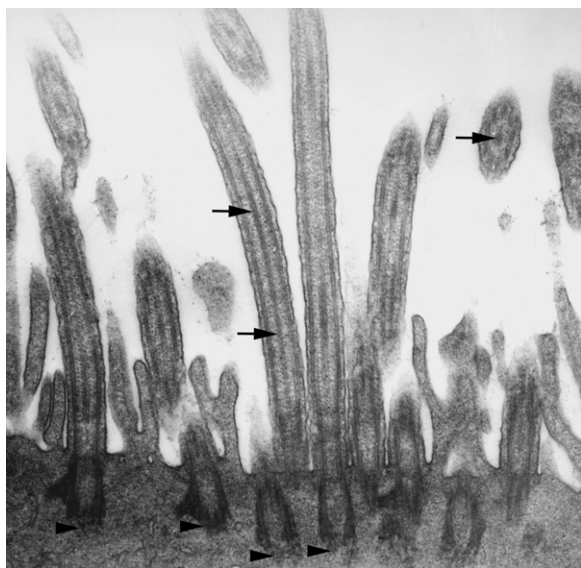


Figure 1 Epithelium of nasal mucosa of the control group with transversely and cross sections of ciliated epithelial cells; typical 9 + 2 pattern with central tubuli (arrows) and peripheral doublets; origin of ciliated cells in blepharoblasts (arrow heads). (Electron microscopy; original magnification $\times 12,000$.)

males and 12 males, at the Department of Otorhinolaryngology–Head and Neck Surgery, Martin Luther University Halle Wittenberg between March 2003 and April 2005. Their ages ranged from 22 to 62 years; mean age was 36.3 years. These patients had overused topical vasoconstrictive nasal spray without benzalkonium chloride for at least 6 months and had chronic nasal obstruction. Patients with a history of allergic or idiopathic rhinitis or acute as well as chronic rhinosinusitis and patients with topic application of glucocorticosteroids were excluded. The patients showed a negative skin test for a standard battery of common allergens and a negative radioallergosorbent test for total and specific IgE. Biopsies from inferior turbinates of 10 patients were taken during septorhinoplasty with turbinectomy as control. The control group consisted of 10 patients (four female, six male; age range, 20 to 51 years; mean age, 32.1 years) without chronic inflammations of nasal mucosa.

Preparation for Histology

The samples were fixed in buffered formalin. After dehydration, the specimens were embedded in paraffin. For morphologic evaluation, the sections were stained with Mayer's hemalum or methylene blue and photodocumented with a lighted microscope.

Preparation for Electron Microscopy

The specimens were immersed in a solution of 3% phosphate-buffered glutaraldehyde (according to Schultz-Karlsson). After a brief washing procedure in buffer and dehydration, the specimens were incubated in unicryl overnight at 4°C. After polymerization, serial ultrathin sections (70 nm)

were cut (Reichert-Jung Ultracut E, Vienna, Austria) and then placed on nickel grids.

Transmission Electron Microscopy

After washing in phosphate buffer, they were refixed in a solution of 1% osmium acid, and after a renewed buffer washing, we performed dehydration in an ascending acetone series. A penetration phase with Durcupan or Araldit was followed by heat polymerization in which the specimens were caused to harden. After preparing semithin cuts and staining with toluidine blue, we selected appropriate tissue areas of inferior turbinate for the electron microscopy. Finally, the preparations were cut ultra thin (Reichert-Ultracut). After double-contrasting with uranylacetate and lead citrate, ultrastructures were photodocumented with a transmission electron microscope (EM 902 A Zeiss, Jena, Germany). Special attention was paid to changes of epithelium, seromucous glands, and the vascular system.

The study was approved by the Institutional Review Board of the MLU Halle Wittenberg and was in accordance with the Declaration of Helsinki.

RESULTS

In specimens of nasal mucosa of the control group, a regular morphology of the pseudo-stratified epithelium with typical 9 + 2 pattern of microtubuli was observed (Fig 1). In all cases of investigated patients with rhinitis medicamentosa, the nasal epithelium showed extensive damaged areas as well as severe hyperplasia and metaplasia (Figs 2 and 3), as compared with the control group (Fig 1). The partly loosened epithelium with numerous vacuoles displayed rounded and edematous changed pleomorphic cells (Figs 3, 4). The intercellular spaces were dilated. The cilia were severely

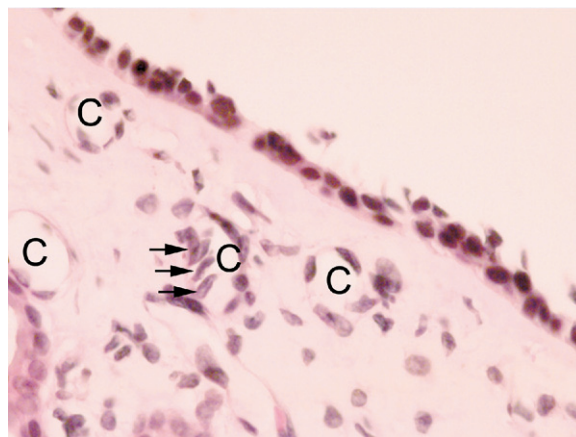


Figure 2 Changes of the ciliated epithelium after long-term medication with topical vasoconstrictors (imidazoles) shows loss of typical epithelial layers and of ciliated cells. The subepithelial edematous mucosa reveals capillaries (C) with prominent endothelial lining (arrows). (Hematoxylin-eosin stain; original magnification $\times 120$.)

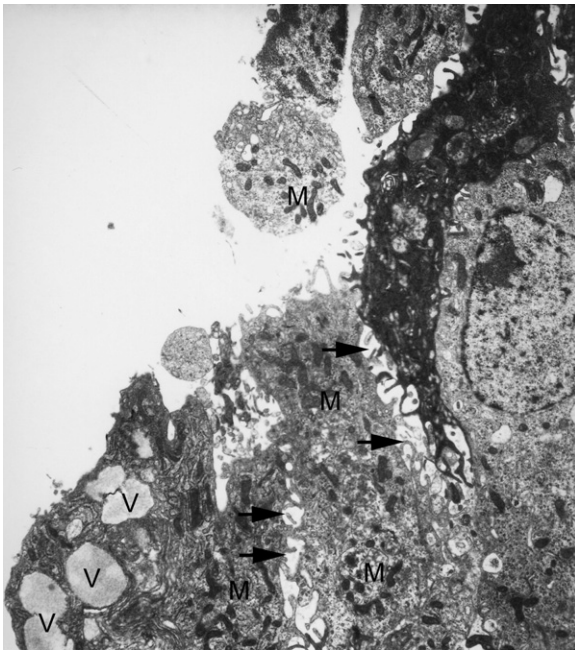


Figure 3 Epithelium of nasal mucosa of patient with rhinitis medicamentosa illustrated under electron microscope shows loss of cilia and damaged epithelial cells; intraepithelial numerous vacuoles (V) and mitochondrias (M); dilated intercellular spaces (arrows). (Original magnification $\times 7,000$.)

damaged. In wide areas of the epithelium, we proved loss of ciliated epithelial cells and dilated intercellular spaces (Figs 2, 3, and 4).

Beneath a thick basal membrane, capillaries and venous vessels showed several ultrastructural changes in contrast to the normal group. In normal nasal mucosa, the capillaries

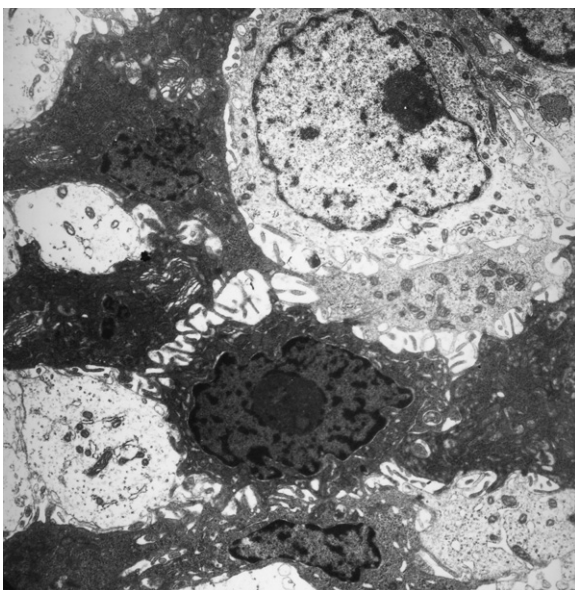


Figure 4 Metaplastic changes of the prismatic epithelial cells into rounded cells. Note the wide intercellular spaces. (Electron microscopy; original magnification $\times 7,000$.)

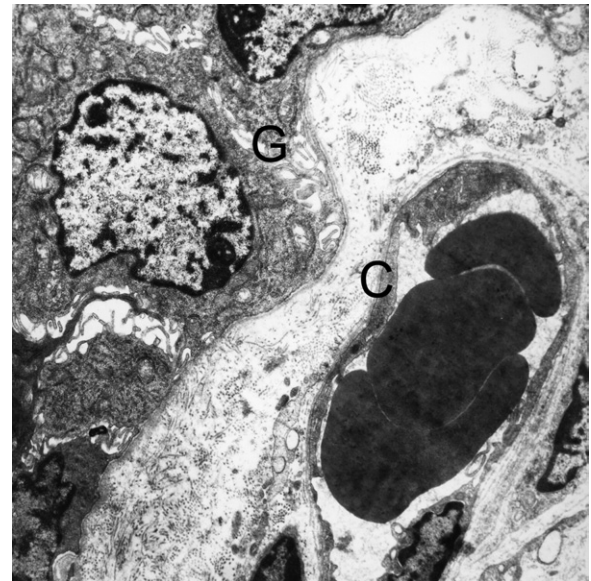


Figure 5 Electron microscopic appearance of normal capillary (C) with 3 red blood corpuscles intracapillary in contact to a seromucous gland (G); normal endothelial lining. (Original magnification $\times 7,000$.)

showed continuously or fenestrated endothelial lining (Fig 5). In rhinitis medicamentosa, prominent endothelial cells and considerable endothelium gaps could be detected regularly in capillaries (Figs 6 and 7). The endothelial cells contain numerous pinocytosis vesicles (Fig 7). Ruptures of the vascular basal membrane were conspicuous (Fig 8). Perivascular and extensive edema of the surrounding connective tissue and numerous concentric proliferated pericytes and pericyte lamellas were displayed (Fig 7). Partial hyperplasia of goblet cells and proliferated seromucous glands were demonstrated. Ultrastructurally pathologic infiltration with eosinophiles or mast cells could not be detected.

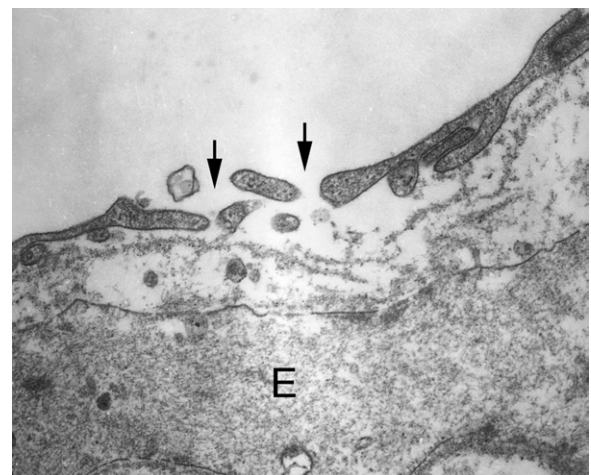


Figure 6 Endothelial lining of a capillary; gaps and ruptures (arrows) of the endothelial cell (E). (Electron microscopy; original magnification $\times 20,000$.)

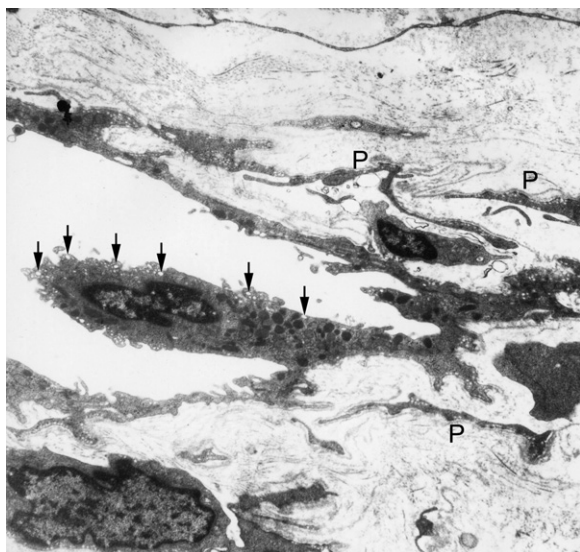


Figure 7 Prominent endothelial cell of a capillary. Note the numerous pinocytosis vesicles (arrows). In the edematous interstitial tissue concentric pericytes (P). (Electron microscopy; original magnification $\times 7,000$.)

DISCUSSION

The topically applied imidazoles oxymetazoline and xylo-metazoline belong to the most frequently prescribed or self-medicated nasal sprays. From the point of view of evidence-based medicine, there is no proof of effectiveness of decongestive nasal sprays or drops in the treatment of acute or chronic sinusitis in the rare existing studies. However, they lead to a subjective soothing of the nasal obstruction, and they are accepted by the patients.¹² Long-term application leads to tachyphylaxis. The application intervals become shorter with only insufficient decongestant effect.^{13,14} The animal model was able to display morphologic changes after naphazoline application.⁷⁻⁹ In the nasal mucosa of rabbits, vasoconstrictions and thrombotic occlusions of vascular lumen and narrowing of arterial vessels respectively could be detected.⁷ These extensive partial obliterations of the resistance vessels could not be found in human nasal mucosa. In contrast to specimens of normal nasal mucosa,¹⁵ morphologic changes of the exchange and capacity vessels were conspicuous. Especially, transmission electron microscopic investigations were able to demonstrate the underlying morphologic changes of nasal mucosa in rhinitis medicamentosa. Because of ruptures and gaps that form in the endothelial lining, both endoluminally as well as of the basal membrane extravascularly, this may lead to extravasation of serum components and accordingly consecutively to mucosa edema. Ultrastructurally increased pinocytosis vesicles in endothelial cells could be observed.

This observation testifies to highly activated cells with an increased exchange function. In addition, the endoluminally prominent endothelium cells indicate toxic damage through imidazoline derivates. Wang and Bu¹⁰ were also able to

detect changes of endothelial cells, such as thickened basal membranes of the capillaries. In contrast to Wang and Bu, however, we found discontinuing basal membranes to extensive gap formations that may assist an interstitial edema formation. According to Graf et al.,^{4,5} positive effects of topical glucocorticosteroides (Budesonid) on the nasal obstruction in patients with rhinitis medicamentosa were reported, as a result of the influence of nasal steroids on the vascular permeability. There is some evidence of formation of a manifest edema of the mucosa after only 6 weeks of decongestant nasal spray use.¹³ A further application of nasal vasoconstrictors cannot influence this edema; it leads to a rebound phenomenon.⁴

Animal experiments demonstrated massive damages of epithelium cells, such as metaplasia and loss of cilia.⁸⁻¹⁰ In particular, oxymetazoline seems to irreversibly inhibit activities of ciliated cells. In literature, different statements were found concerning the cytotoxic effects of benzalkonium chloride, a preservative that is used less frequently in the past years because of the use of preservative-free nasal sprays.

In vitro studies had shown degenerative effects of benzalkonium chloride on nasal mucosa.^{4,14,16,17} In contrast to these investigations, in vivo studies were unable to demonstrate significant damages of nasal mucosa due to benzalkonium chloride.^{17,18} It could be assumed that a protective effect exists as a result of mucosal barrier action, variable dilution of benzalkonium chloride, enzymatic degradation, and pH effect on benzalkonium chloride activity.¹⁷ These data suggest the safety of this preservative when used at appropriate concentrations. Patients of this study have not used nasal decongestants with benzalkonium chloride.

Ultrastructurally, a considerably layer-disturbed epithelium with hyperplastic and metaplastic changes as the morphologic correlate to ciliary toxic effects of decongestive nasal sprays was observed. In wide areas, loss of cilia and

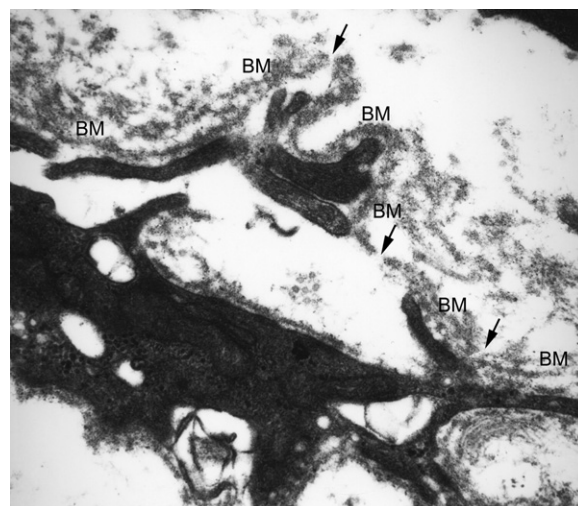


Figure 8 Electron microscopic image in an overview magnification. The basal membrane (BM) shows continuity interruptions and gap forming (arrows). (Original magnification $\times 30,000$.)

dilated intercellular spaces was found. It could be assumed that damages of epithelial cell, enlargement of intercellular spaces, and particularly hyperplasia of goblet cells contribute to dysfunctions of mucociliary clearance. In contrast, Lin et al¹¹ assumed the hyperplasia of goblet cells and increased seromucous glands as causation for nasal obstruction. In contrast to these assumptions, we attribute damages of epithelial cells and changes of endothelial lining to the underlying pathogenesis in rhinitis medicamentosa.

According to our observations in rabbits, accumulation of inflammatory cells, such as eosinophiles or mast cells in patients with allergic rhinitis, could not be detected.^{7,19} The present ultrastructural findings show considerable pathologic changes of the nasal mucosa after long-term use of decongestant nasal sprays. For a prophylaxis of rhinitis medicamentosa, it is necessary to extensively counsel and inform the patients before they start taking these nasal vasoconstrictors. According to Kloecker et al,¹⁴ the addition of dexamphenol seems to be suitable to diminish the ciliary toxic as well as cytotoxic effects of imidazoles. With respect to mucociliary clearance, preservative-free dexamphenol nasal spray seems to be better than dexamphenol nasal ointment. In order to avoid additive ciliary toxic effects, preservative-free nasal decongestants are recommended.^{4,14,16} In cases of rhinitis medicamentosa, the patients must immediately and completely stop taking topical vasoconstrictive nasal sprays to allow the harmed nasal mucosa to recover. For treatment of the causative nasal disease, topical or oral glucocorticosteroids are most effective.^{4,5,8} The anti-edematous effect of glucocorticosteroids has a demonstrable impact on the edema of nasal mucosa. According to Minshall et al²⁰ glucocorticosteroids (mometasone furoate) could lead to refinement of epithelial integrity. Furthermore, increased ciliated cells and a reduction of metaplastic areas were observed. Mastruzzo et al²¹ demonstrated positive effects of intranasal budesonide on epithelial remodeling. The restitution of the epithelium seems to influence by the induction of TGF- β , a growth factor. Graf et al⁵ were able to achieve a success rate of 100% within 7 weeks with the use of budesonide nasal spray in patients with severe rhinitis medicamentosa.

After withdrawal of local vasoconstrictors, topical glucocorticosteroids should be used to decrease the symptoms, such as obstruction of nasal mucosa, in patients with rhinitis medicamentosa.

We wish to thank Ms Vogtlaender and Ms K. Linde for the technical assistance.

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