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Reversibility and clinical relevance of morphological changes after nasal application of ephedrine nasal drops 1%

Erik Bechgaard [a,*], Erling Bindseil [b], Morten Bagger [a], Hanne Wulff Nielsen [a,c]

[a] *Royal Danish School of Pharmacy, Department of Pharmaceutics, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark*
[b] *The Royal Veterinary and Agricultural University, Department of Pharmacology and Pathobiology, Bülowsvej 13, DK-1870 Frederiksberg C, Copenhagen, Denmark*
[c] *Peptech (Europe) A/S, Herredsvejen 2, DK-3400 Hillerød, Denmark*

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Abstract

To predict the toxicity of nasal formulations, various in vitro and in vivo techniques have been used. Many of these techniques are very sensitive and it is a general problem to extrapolate the results to the clinical situation. The aim of the present study was to establish a clinically well known nasal formulation, Ephedrine Nasal Drops 1%, DAK 63 (EBE), as a relevant reference for other nasal formulations with respect to histological changes to and reversibility of the nasal mucosa after repeated short-term nasal application to rabbits. This ephedrine formulation also contains the well known local irritants, benzalkonium chloride and EDTA, which is why it is abbreviated to EBE. Seventy five μl was applied in one nasal cavity of rabbits ($n = 3$) four times per day for 1 week, while the other cavity served as a control. Twelve rabbits were divided into four groups and were sacrificed at 4 h and 1, 7 and 21 days after last nasal application, respectively. The macro- and microscopical changes of the nasal mucosa were recorded. Except for minor greyish exudates seen at 4 h, and a slight congestion of the mucosa seen at 4 and 24 h after application, there were no gross changes of the nasal mucosa. The microscopical examination, however, showed an extended infiltration of the mucosa by eosinophils, a general inflammatory reaction and a pronounced atrophy and disorganisation of the epithelium, which was furthermore void of goblet cells and cilia. These microscopical changes were seen after 4 h and, to a minor extent, 24 h after application. After 7 days, no changes could be found, indicating that they were reversible in less than 1 week. It is concluded that EBE may be a good reference in the predictive testing of local toxicity, with respect to a cost/benefit evaluation of nasal formulations, meant for acute or short-term treatment. © 1997 Elsevier Science B.V.

Keywords: Ephedrine; Nasal drops; Morphological changes; Nasal application

* Corresponding author.

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1. Introduction

In recent years, much attention has been paid to the systemic delivery of drugs by the nasal route (Chien, 1994). The ease of administration and fast absorption without first pass metabolism makes nasal application an attractive alternative to the parenteral route. However, with nasal application it is very important to evaluate and reduce the local toxicity at the site of application. Therefore, it is of great importance to know the clinical relevance of various predictive tests for local toxicity.

To predict the toxicity of nasal formulations, various in vitro and in vivo techniques have been used (Gizurarson, 1992; Pereswetoff-Morath et al., 1996) including measurement of the mucociliary clearance (CBF) (Hermens and Merkus, 1987; Gizurarson, 1990), of electrophysiological properties, e.g. using chamber technique (Bechgaard et al., 1993; Vermehren et al., 1996), of erythrocyte haemolysis (Hirai et al., 1981), influence on the viability of cell cultures (Jørgensen et al., 1993), and histological studies (Björk et al., 1991; Chandler et al., 1994; Bindseil et al., 1995). The techniques are also used in combination with scanning electron microscopy (SEM) and evaluation of drug bioavailability or with measurement of release of biochemical markers from the nasal mucosa (Marttin et al., 1995). In establishing the actual local toxicity of nasal drug formulation, most of these predictive techniques, i.e. measurement of CBF in vitro (Romeijn et al., 1996), is probably a too sensitive approach. Primarily, these methods give valuable information on the toxic potency of a nasal drug or excipient. There is a need for further correlations to studies where the clinical situation is resembled.

To establish such correlations, nasal drugs or formulations which are well known from clinical practice may be tested and compared with other substances with respect to local toxicity. The use of Ephedrine Nasal Drops 1% (EBE), may be a relevant reference, as this formulation has been used for many years as a nasal decongestant and since it is known to be irritating to the nasal mucosa. The irritative effect of the formulation, EBE, is probably not caused by ephedrine alone, but also by the preservative benzalkonium chloride 0.01%, which has been shown to exert histological changes in rabbit nasal mucosa (Marttin et al., 1996) and by EDTA, well known for disruption of epithelial tight junctions (Lee, 1991). Pure ephedrine solutions, 0.5 and 1.0%, have also been shown to cause a decrease in the ciliary beat function (CBF) in vitro (Su and Po, 1993). Another in vitro study on CBF has shown the effect of ephedrine 0.5% to be reversible within approximately 1 h (van de Donk et al., 1982).

Ephedrine solutions for use in the nasal cavity is still accepted, e.g. the British Pharmacopoeia (Vol. 2, 1993, p. 896) and USP (23, 1995, p. 594). In Denmark, however, EBE was withdrawn in 1994 primarily due to the introduction of new drugs with a prolonged effect and with less systemic and local side effects.

The local irritation from EBE is well known, which is the reason why the use of the formulation has been restricted to five daily applications for 1 week. A registration of the toxic effect on the nasal mucosa after such a dosage regime with EBE is therefore clinically relevant and may serve as a toxicity reference for the correlation of other drugs, absorption enhancers or vehicles for use in the nasal cavity.

Therefore, the effects of single applications in vivo of EBE were studied 4 h after application by a combined macro- and microscopical investigation of the rabbit nasal septae and conchae (Bindseil et al., 1995). Surprisingly, a mild toxicity was observed after only one application indicated by histological changes.

The aim of the present study was to establish EBE as a clinically relevant reference for nasal formulations with respect to the histological changes of the nasal mucosa and their reversiblity after repeated short-term nasal applications to rabbits. The study was intended to be relevant to the discussion of nasal formulations, with or without the use of absorption enhancers.

2. Materials and methods

2.1. Materials

Ephedrine hydrochloride, Na-EDTA and NaCl of Ph. Eur. (2nd Ed.) quality and benzalkonium chloride of Ph. Nord. 63 quality were obtained from Nomeco (Copenhagen, Denmark). Destilled water of Ph. Eur. (2nd Ed.) quality was used throughout the experiments.

2.2. Animals

Twelve male New Zealand White rabbits were obtained from Hvidesten (Denmark). They were housed individually with free access to water and given approximately 100 g Altromin No. 2123 from Chr. Pedersen (Ringsted, Denmark) daily. The rabbits were approximately 1 year old and weighed 3.6–4.7 kg at the beginning of the experiments.

2.3. Nasal formulation

The nasal formulation, EBE, consisted of 1.0 g ephedrine, HCl, 10.0 mg benzalkonium chloride, 50.0 mg Na-EDTA and 0.6 g NaCl dissolved in 98.5 g destilled water as described for Ephedrine Nasal Drops 1% in DAK 63. The formulation was stored at 4°C.

2.4. Experimental design

Seventy five μl of EBE was administered with an Eppendorph Multipipette® to the right nasal cavity of the conscious rabbits four times daily for 1 week. The animals were observed closely during the dosing period, as unnormal behaviour of the rabbits was not accepted.

The rabbits were killed 4 h and 1, 7 and 21 days after the last nasal application by anaesthesia with a sodium pentobarbital injection (50 mg/ml) and exsanguination by an incision in the carotid arteries. Immediately after exsanguination the nasal cavities were opened by removing the nasal and maxillary bones.

2.5. Post mortem procedures

2.5.1. Gross examination

After removal of the nasal and maxillary bones, the nasal mucosa was inspected and recordings were made of the gross appearance.

2.5.2. Light microscopy

After opening of the nasal cavity, the septum and the ventral nasal conchae were removed separately and fixed in 10% neutral, buffered formalin. After fixation the conchae were decalcified in a 14% solution of ethylenedinitrotetraacetic acid for at least 2–3 days, and each septum was transected at three different levels to obtain tissue blocks from the anterior, middle, and posterior regions.

The decalcified conchae and the septal tissue blocks were processed routinely by imbedding in paraffin and sectioning at 5 μm. The conchae were sectioned longitudinally.

The sections were stained with haematoxylin-eosin, with Luna's stain for eosinophilic granulocytes, with periodic acid-Schiff reagent (PAS) for ground substance, and with Van Gieson's stain for collagen.

3. Results

A summary of the results appears in Table 1, where score values for various histological features are displayed. A detailed description is as follows:

3.1. Clinical signs

No clinical signs of respiratory disease, e.g. dyspnoea and exudative discharge from the nares, were observed in any of the rabbits. The activity and food consumption was also normal, and no signs of discomfort were observed.

3.2. Gross examination

At 4 h, minor greyish exudates were found in the right, applicated nasal cavity between the ventral conchae and the septum in 2 of the 3 rabbits. No changes were observed in the left,

Table 1
Score of main histopathological features in the nasal septae of rabbits ($n = 3$ in each group) at various times after repeated (1 week 4 times/day) nasal application of Ephedrine Nasal Drops 1% (EBE) in the right nasal cavity (The left cavity was used as a control)

Histopathological feature	Control	4 h	24 h	7 days and 3 weeks
Exudate on mucosa	0	+	0	0
Cellular infiltration in epithelium	0	++	0	0
Inflammation in lamina propria	0	+	0	0
Epithelial atrophy	0	+++	0	0
Epithelial hypertrophy	0	0	+++	0
Epithelial metaplasia	0	+++	+++	0
Loss of goblet cells and cilia	0	+++	+++	0

0, not present or negligible; +, slight; ++, moderate; +++, marked.

untreated nasal cavity. The septum appeared normal in all three animals, whereas there was a slight congestion of the right conchae in all three animals.

At 24 h a slight, bilateral congestion of the treated conchal mucosa was observed in all three animals.

At 7 and 21 days, no changes of the treated nasal mucosa were observed.

In the left nasal cavities, serving as untreated controls, no macroscopical changes of the nasal mucosa were observed.

3.3. Light microscopy

At 4 h, the right applicated side of the nasal cavity of all three animals, scattered strands and clumps of exudate with fibrin, neutrophils, eosinophils and desquamated epithelial cells were seen on the conchal mucosa (Fig. 1). Particularly in the rostral parts of the conchae, the epithelium was slightly to heavily infiltrated of neutrophils and eosinophils, and patchy epithelial disorganisation and necrosis were seen. Inflammatory reactions dominated by eosinophils, oedema, and hyperaemia were seen in the lamina propria. Many small blood vessels contained eosinophils. There appeared to be a slight increase in the number of activated osteoblasts along the bony spicules.

The septal epithelium of the right, treated nasal cavity showed, in all three regions, a marked patchy atrophy or metaplasia (substitution of one type of cells for another). The atrophy could be so pronounced as to leave an unilayered, flat epithelium almost like an endo- or mesothelium (Fig. 2). The metaplastic epithelium was of the same thickness as the epithelium on the untreated, normal side, but consisted of a layer of disorganised epithelial cells without a distinct, regular basal layer (Fig. 3). Regardless of whether the epithelium was atrophic or metaplastic, it was almost void of goblet cells and cilia.

Either type of changed epithelium was infiltrated of eosinophils which were also seen in

Fig. 1. Marked cellular exudate (E) on the conchal mucosa in the right nasal cavity of a rabbit 4 h after repeated nasal application of Ephedrine Nasal Drops 1% DAK 63 (EBE), for 1 week 4 times/day. Conchal epithelium (C) and bony spicule in conchal lamella (B). Bar = 75 μm.

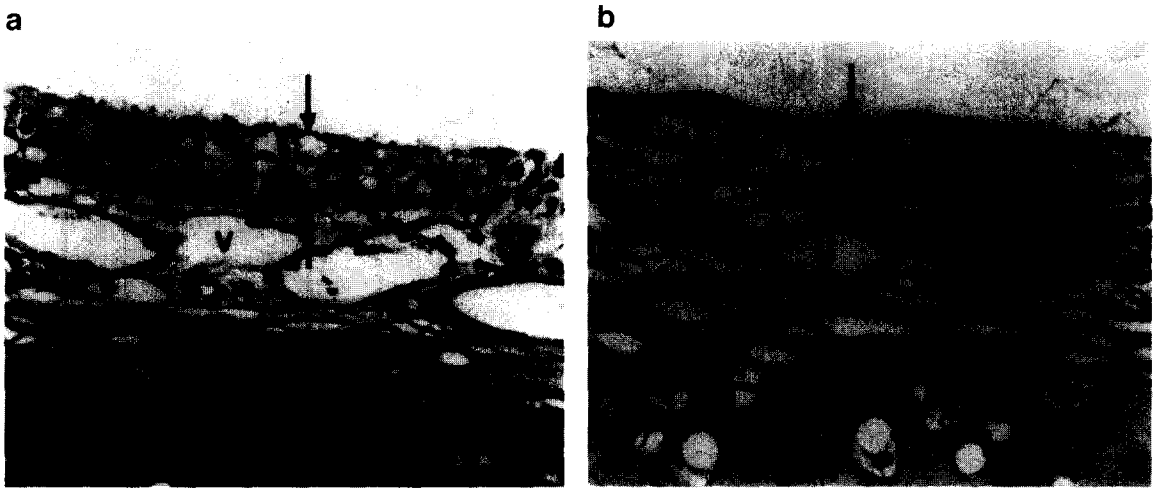

Fig. 2. (I) Normal features of septal respiratory epithelium (between arrows) of the left, untreated side of the nasal cavity of a rabbit. Vascular plexus (V) in lamina propria, goblet cells (G) and septal cartilage (S). The vague zone observed on the luminal surface of the epithelial cells is a brim of cilia. Bar = 75 μm. (II) Marked atrophy of septal, respiratory epithelium (arrow) in the right, treated side of the nasal cavity of a rabbit 4 h after repeated (1 week) nasal application of EBE. Note lack of goblet cells and cilia. Vascular plexus (V) and septal cartilage (S). Bar = 75 μm.

the lamina propria and in the lumen of small blood vessels. Minute strands of exudate containing fibrin, eosinophils, and desquamated epithelial cells were occasionally seen on the surface of the epithelium.

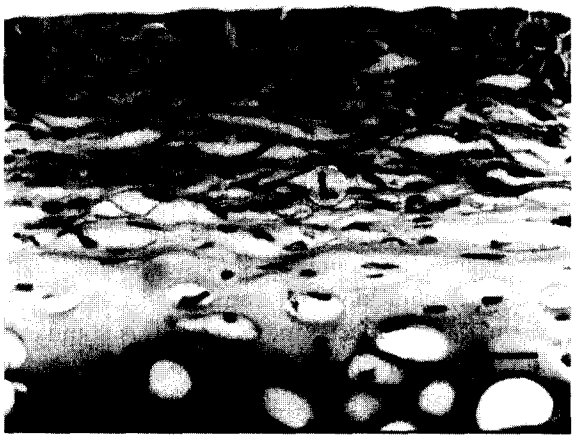

Fig. 3. Metaplastic, septal, respiratory epithelium (E) in the right, treated side of the nasal cavity of a rabbit 4 h after repeated nasal application of EBE. The epithelium is of the same height as normal respiratory epithelium (Fig. 2I), but it is completely void of goblet cells and cilia. Septal cartilage (S) and lamina propria (L). Bar = 75 μm.

At 24 h, the conchal epithelium in the right nasal cavity showed less of the histopathological features described in rabbits sacrificed at 4 h, but they were principally identical.

The vestibular squamous epithelium of the septum in the right side was hyperplastic (increase in the number of cells) compared with the epithelium of the left side (Fig. 4). The epithelium of the respiratory and olfactory regions was metaplastic in places as described in the animals sacrificed at 4 h, and was almost void of goblet cells and cilia. A few scattered mitoses were observed in the epithelium, but epithelial atrophy was not a feature in this group of animals.

The inflammatory reaction in the lamina propria was negligible, and only a few eosinophils were detected in the epithelium and the underlying tissues.

At 7 and 21 days, no essential pathological changes were observed in the right, applicated nasal cavity in any of the rabbits.

The nasal mucosa of the untreated left nasal cavities, displayed no pathological changes in any of the animals. Scattered eosinophils and minor foci of lymphoid tissue, which are normal features in the nasal mucosa of animals, were occasionally observed.

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