Anatomical Structure and Surface Epithelial Distribution in the Nasal Cavity of the Common Cotton-Eared Marmoset (Callithrix jacchus)

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Abstract: To validate use of the common cotton-eared marmoset (Callithrix jacchus) in inhalation toxicity studies, its nasal morphology was examined. The nasal turbinates each consisted of one maxilloturbinate and one ethmoturbinate; these were more planar in structure than the comparable structures of rodents or dogs. The nasal cavity epithelia comprised squamous epithelium (SE), nasal transitional epithelium (NTE), respiratory epithelium (RE) and olfactory epithelium (OE), listed in order of occurrence from anterior to posterior positions. NTE was distributed as a narrow band lying between SE and RE. OE was limited to the dorsal part of the cavity, which was structurally similar to that of the macaque or man. Overall, this study revealed structural the similarity of the whole nasal cavity in the marmoset to that of macaques or humans. Prediction of nasal cavity changes in man based on extrapolation from experimentally induced changes in the common marmoset therefore seems likely to be feasible, making it a useful animal model for inhalation studies.

Key words: inhalation, marmoset, nasal cavity, turbinate

Introduction

Recently, inhalation toxicity studies have become increasingly required to assess the safety of environmental chemicals, agricultural chemicals and medicines. The nasal cavity, as an entrance to the respiratory tract, plays an important role in protection against harmful exposures. Inspired air is heated and humidified in the nasal cavity, water soluble gases and vapors are absorbed into the nasal epithelium [3, 6, 20, 26], and particles in inspired air are deposited in the nasal cavity then removed [4, 7, 8, 12, 16, 23]. Therefore, the nasal cavity can be considered the first site to be affected by inhaled chemicals. For extrapolation to human nasal toxicity from animal inhalation experiments, laboratory rodents and dogs are not always suitable test species because of differences in nasal cavity structure. On these grounds, nonhuman primates are often recommended for use in inhalation studies [11]. We have attempted to use the common cotton-eared marmoset
(Callithrix jacchus) for inhalation toxicity studies because of the advantages conferred by their small size, low maintenance costs and ease of handling. There are many reports about the nasal cavity structure of humans [5, 15, 22, 27], dogs [2, 21, 25], laboratory rodents [1, 9, 25] and monkeys [17, 18, 24, 25], but, to the best of our knowledge, there are no comparable reports dealing with marmosets. This study addresses the nasal anatomy and structure (including epithelial distribution) in marmosets.

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**Materials and Methods**

*Animals:* Common cotton-eared marmosets were purchased from a commercial breeding company (CLEA Japan Inc., Tokyo, Japan) which had derived its stock from the Central Institute for Experimental Animals (Kanagawa, Japan). The animals were housed individually in metal cages (30 × 60 × 75 cm). The animal room was maintained at a temperature of 25–28°C, with a relative humidity of 40–60%, ventilation 15 times/hr, and a 12 hr light-dark cycle. Each animal was fed daily with 15–20 g of a basic diet mixed with warm water, and allowed free access to tap water. The basic diet consisted of a standard pellet diet (expanded s-type feed for small primates, Oriental Yeast Co. Ltd., Tokyo, Japan), supplemented with honey, vitamin D₃ and vitamin C. The animals were treated with appropriate care and respect, in accordance with the Guidelines for Animal Experimentation of our laboratory, Mitsubishi Chemical Safety Institute Ltd.

*Anatomical and histological examination:* Nasal tissues were obtained from 5 males and 2 females, 15 to 48 months of age, which had been used as untreated control animals in a non-inhalation toxicity study. The animals were anesthetized with sodium pentobarbital (intraperitoneally injected at a dose of 30 mg/kg body weight) and the euthanized by exsanguination via the abdominal aorta. The head, after removal of brain and eyeballs, was immersed in a large volume of 10% neutral phosphate-buffered formalin until examination. Subsequent to removal of the integument and mandible, the entire nasal cavity and calvarium were decalcified with 15% formic acid formalin for 5 to 7 days. After decalcification, the nasal structures of 2 males were macroscopically examined in the longitudinal plane. For histological examination, nasal cavities obtained from 3 males and 2 females were cut transversely into 4 blocks, as shown in Fig. 1. Anatomical positions of the cutting points were as follows: anterior to the cynodont, anterior to the second premolar, anterior to the first molar and about 4 mm posterior to the second molar. Each block was embedded in paraffin. Sections 5 μm-thick were collected every 100 μm through each block, and stained with hematoxylin and eosin (H.E.). Representative sections were stained with Alcian blue pH 2.5/periodic acid-Schiff’s solution (AB/PAS). By light microscopy, the nasal epithelium was classified as squamous, nasal transitional, respiratory or olfactory according to definitions of Harkema [10].

To estimate the distribution of each epithelium quantitatively, representative sections of blocks from the 3 males were photographed. The perimeter in the nasal cavity covered by various epithelia was marked on the

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**Fig. 1.** Diagrammatic representation of the marmoset nose to show levels where histological sections were made. A, side view of nasal cavity with the nasal septum removed; B, ventral view of hard palate region with the lower jaw removed. Vertical lines indicate cutting points: 1, anterior to the cynodont; 2, anterior to the second premolar; 3, anterior to the first molar; 4, about 4 mm posterior to the second molar.
photographs. The marked photographs of each section were computerized. The perimeter covered by each type of epithelium was traced and the length was measured with an image analyzer (IPAP, image processor for analytical pathology; Sumika Technos, Osaka, Japan).

Results

Gross anatomy: A distinct thickening was observed in the mid-part of the nasal septum from the level of the second incisor to that of the cynodont in block 1 (Fig. 2). Each nasal turbinate was composed of one ethmoturbinate and one maxilloturbinate (Figs. 2 and 3). The maxilloturbinate arose from the maxillary bone at the lateral wall of the nasal cavity and extended to the anterior part near the nasal vestibule. The ethmoturbinate arose from the ethmoidal plate at the dorsolateral wall and extended to the upper part of the first premolar. The turbinates had no definite ramification.

Distribution of the nasal epithelium: The nasal epithelium was composed of squamous epithelium (SE), nasal transitional epithelium (NTE), respiratory epithelium (RE) and olfactory epithelium (OE), listed in order of occurrence from anterior to posterior regions (Fig. 4). SE was composed of 5 or more layers of squamous cells (Fig. 5-A). No hair follicle was observed in the area covered by SE. Melanin pigments in the basal layer disappeared gradually towards the posterior. The keratin layer also disappeared just prior to changing to NTE. The SE was covered from the nasal inlet to the first premolar or the cynodont. The NTE, corresponding to nonciliated respiratory epithelium, was characterized by nonciliated cuboidal or columnar surface cells and a small number of mucous-containing-cells (Fig. 5-B). This area was slightly depressed, because the epithelium was thinner than the SE and RE. The NTE was distributed in a narrow area between the SE and RE,
first appearing on the dorsal aspect of the front vestibule and extending to the ventral aspect of the posterior vestibule. The most conspicuous cells in the RE were tall ciliated columnar surface cells and mucous containing columnar surface cells, or goblet cells (Fig. 5-C). Nuclei were crowded in the lower two thirds of the epithelial layer; this feature resembled a pseudostratified epithelium. Nasal glands were seen in the propria of the RE. The RE covered the broadest area in the nasal cavity and extended to the nasopharyngeal tract. The OE comprised an upper single layer of sustentacular cells, several (approximately 5 to 7) layers of olfactory cells, and a single lower layer of basal cells (Fig. 5-D). The epithelial surface was covered with secretions from the olfactory (Bowman's) gland. Olfactory glands and bundles of nerve fiber were seen in the propria. The OE was distributed on the upper part of the RE, lining only the upper third of the lateral wall and the septum. Table 1 shows the length percentage of the mucosal surface of each epithelium. RE was the most conspicu-
Table 1. Surface length of the nasal mucosa: percentage of each epithelial type

<table>
<thead>
<tr>
<th>Block number</th>
<th>Type of epithelium</th>
<th>Squamous</th>
<th>Nasal transitional</th>
<th>Respiratory</th>
<th>Olfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>22.7 ± 12.9</td>
<td>22.9 ± 5.7</td>
<td>54.5 ± 13.8</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>70.8 ± 8.7</td>
<td>29.2 ± 8.7</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>70.4 ± 7.6</td>
<td>29.6 ± 7.6</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Value are expressed as mean of 3 males ± standard deviation.

Discussion

The purpose of the present study was to characterize the anatomical structure and epithelial distribution in the nasal cavity of the common cotton-eared marmoset (*Callithrix jaccus*) for use in inhalation toxicity studies.

On gross anatomical observation, a distinct thickening of the nasal septum was observed. Such a thickening was reported in the macaque and has also been shown in figures representing nasal structures of the tree shrew, gibbon, baboon and man [17, 22], but this thickening has not been reported in rats or dogs. Thickening of the nasal septum may therefore be a structure characteristic of primates. The nasal turbinate of the marmoset were each composed of one ethmoturbinate and one maxilloturbinate, the ramification of which was unclear. The macaque has two turbinates similar to the marmoset, for which the ramification is also unclear [11]. Although humans [22] have 2 ethmoturbinates and 1 maxilloturbinate, the structure is very simple, and ramification of the turbinate is also unclear, so a general similarity to the situation in marmosets and macaques may be claimed. By contrast, rats (which are widely used in inhalation experiments) have 4 nasal turbinates: a nasoturbinate, maxilloturbinate, endoturbinate and ectoturbinate, and these have complex ramifications [28]. Beagle dogs also have 4 nasal turbinates, and the structure is more complex than that of rats [2].

The NTE of the marmoset occurred in the dorsal portion of the nasal vestibule, and extended to the ventral aspect of the posterior portion. It was reported that in the macaque the NTE extended from the ventral aspect of the nasal vestibule to the dorsal aspect of the posterior [11]. In rats, the distribution of NTE is similar to that in the marmoset. The reason for these inter-species differences in NTE distribution is not clear. The OE is distributed only in the upper third of the nasal cavity of the marmoset (Fig. 4). The percentage of surface length of mucosa covered by OE was approximately 30% in representative sections of blocks 2 and 3 in the marmoset. Gross et al. reported the percentages of OE area in rats and mice were about 50 and 45% [9]. According to the data on the surface area of the nasal cavity of various species summarized by Adams [1], dogs and rodents have a higher percentage of OE than monkeys and man. Rats and dogs are classified into macrorrhombic animals, because these species have many ramifications of the nasal turbinate and a high relative surface area of OE. On the other hand, the results of the present study indicate that the marmoset may be classified as a microsomatic animal, similar to the macaque and man.

In inhalation toxicity experiments, the area of nasal epithelium injured differs with different inhaled chemical toxins, and also according to the animal species [13, 14]. The latter differences are considered to be related to the differences in airstream capacity of the nasal cavity [19], and to differing distributions of xenobiotic metabolizing enzymes in this cavity [11]. The pattern of the nasal airstream depends on nasal structure. Therefore, when considering the fate of inhaled materials in the nasal cavity, the degree of similarity between man and selected laboratory animals in nasal anatomy is an important factor affecting extrapolation of animal data to humans. The macaque is recommended for use in inhalation studies because of
the similarity of its nasal cavity in structure to that of man [11], but such studies with macaques need to use specialized inhalation systems. Our study suggests that the marmoset has a nasal cavity structure comparable to that of man and macaques. Because marmosets are similar in size to rats, the inhalation system widely used for rats can be used for the marmoset. In addition, handling the marmoset is easier than handling the macaque. It is concluded that it is appropriate to use the marmoset for inhalation studies intended to permit evaluation of the human health effects of inhaled materials.

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References