Analysis of Lower Airway Inflammation in a Rabbit Model of Acute Rhinosinusitis

Adin Selcuk · Ozgur Akdogan · Seren Gulsen Giray ·
Kursat Murat Ozcan · Ibrahim Ozcan ·
Huseyin Dere · Serdar Ensari · Candan Ozogul

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Abstract This study aimed to investigate the association of inflammatory changes of upper and lower airways in a rabbit model of acute rhinosinusitis. The study included six adult albino rabbits. The sinuses of one animal were injected with saline solution and the animal was served as sham control. Other animals were implanted with intranasal *S. aureus* soaked-absorbable gelatin sponge. Acute rhinosinusitis was induced and subjects were sacrificed at the end of the second week. Tissue samples from all levels

of the airway were obtained. They were evaluated for the presence of inflammatory changes histologically. A scoring system for airway inflammation was used for quantitative assessment of the degree of inflammation. Structural changes in the epithelial and stromal layers of the upper and lower airway structures were analyzed, as well. The animal of which the sinuses were injected with saline solution developed neither acute rhinosinusitis nor lower airway inflammation. In contrast, the animals in which acute rhinosinusitis was induced demonstrated significant upper and lower airway inflammation histologically. Inflammatory changes ranged from engorgement of blood vessels and polymorphonuclear cell proliferation within the capillaries, in the perivascular tissue of the epithelium or in the lamina propria and to epithelial disruption. Nasal airway inflammation scores (2.86 ± 1.81) were significantly higher than lower airway scores (1.36 ± 0.77), ($P < 0.01$). We obtained a generalized mucosal inflammatory response against localized bacterial inflammation in a rabbit model of acute rhinosinusitis, confirming the suggestion of ‘one airway—one disease’ from a bacterial infection point of view.

A. Selcuk · O. Akdogan · K. M. Ozcan · I. Ozcan · H. Dere ·
S. Ensari

Ankara Numune Education and Research Hospital, 4th ENT
clinic, Ankara, Turkey
e-mail: drozgurakdogan@gmail.com

K. M. Ozcan
e-mail: kursatmuratozcan@gmail.com

I. Ozcan
e-mail: ibrozcan@gmail.com

H. Dere
e-mail: hdere38@yahoo.com

S. Ensari
e-mail: ensariserdar@gmail.com

S. G. Giray
Histology and Embryology Department, Faculty of Medicine,
Gazi University, Ankara, Turkey
e-mail: serengn@yahoo.com.tr

C. Ozogul
Histology and Embryology Department, Faculty of Medicine,
Dokuz Eylul University, Izmir, Turkey
e-mail: candan.ozogul@deu.edu.tr

A. Selcuk (✉)
50. Sok 1/10, 06500 Ankara, Turkey
e-mail: sadin27@yahoo.com

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Introduction

There is anatomic continuity between the upper and lower airway. Both components of the airway act together as a single physiologic unit showing similar reactions to noxious stimuli. The upper airway shares many properties with the lower airway suggesting the concept of ‘united airway disease’ [1, 2].

Interactions between the upper and lower airway have been extensively studied in patients with allergic rhinitis and asthma [2–4]. But there is only a few studies showing the common inflammatory reaction of airways to local persistent infection of any part of the airway. There is little definitive information about either the contribution of rhinosinusitis to the pathogenesis of bronchiolitis or the effects of chronic pulmonary diseases on upper airways, as well [5–8].

This study was designed to develop lower airway inflammation in a rabbit model of acute bacterial rhinosinusitis and to determine if acute bacterial rhinosinusitis and lower airway responsiveness are interrelated.

Material and Methods

Experimental Animals

This study included six healthy adult male albino rabbits of the same age (six months) and weight (900–1300 g). The experimental protocol and animal-care procedures were approved from Hacettepe University Animal Care and Use Committee and two weeks were allowed for adaptation before the experiments were begun.

Experimental Design and Operating Procedure

All animals were anesthetized intramuscularly with ketamine HCl (100 mg/kg, Ketalar flacon 10 ml, Parke-Davis, New Jersey) and xylazin HCl (10 mg/kg, Rompun flacon 10 ml, Parke-Davis, New Jersey). The sinuses of one animal were injected with saline solution and the animal was served as sham control. It was euthanized after two weeks and sinonasal and lower airway structures of the subject were served as sham controls. For rest of the procedures, 0.05 ml of solution containing 2×10^7 CFU of *Staphylococcus aureus* soaked in absorbable gelatin sponge (Spongostan, Johnson & Johnson Medical Limited, Gargrave, Skipton, UK) was inoculated into the nasal cavities of five animals bilaterally to induce acute rhinosinusitis [9]. The subjects were euthanized at the end of the second week in order to examine the inflammatory changes in the sinonasal mucosa and the lower airway structures. The animals were euthanized with a lethal dose of 10% chloral hydrate.

Histological Processing

Post sacrifice, the subjects were decapitated, middle turbinates and lateral nasal walls of both sides were isolated. Tissue samples from middle turbinates, trachea and both lungs were obtained from each animal. Tracheal sections

were taken at an upper level (approximately 2 mm below the larynx) and at a lower level (just above the carina). Additional lung sections were taken from the level of the mainstem bronchus, proximal airways and distal airways. The specimens were fixed in phosphate buffered 2.5% glutaraldehyde, pH 7.4. The material was further processed for light microscopy and histological analysis as follows: The mucosae of the maxillary, ethmoid and sphenoid sinuses were harvested. All the mucosa samples from sinuses and the tissue samples from middle turbinates, trachea and lungs were mounted in paraffin blocks. The material was sectioned as 1 μ m thick perpendicular to the epithelium, with intervals of 650 μ m, encompassing the whole epithelium. 10–15 slices were obtained from each material; the materials were stained with haematoxylin and eosin. Then the sections were examined under light microscopy.

Morphological Analysis

Specimens were examined microscopically under high power, and cell type and number per high-power field were recorded. In every surgical specimen, the type of epithelium and the mean number of polymorphonuclear leucocyte (PMN) cells were calculated in $\times 40$ high-magnification field in ten areas on light microscopy. Each section was scored for inflammation as zero, half, one, two, three and four depending on the presence and extent of PMN cell infiltration and epithelial damage, [10] (Table 1). Scores for each slide were summed and divided by the number of fields examined to give an average score per airway level.

Statistical Analysis

The statistical analyses were performed with SPSS v.13.0 and $P \leq 0.05$ were accepted as significant. The statistical significance of the results was tested with the paired *t*-test. For comparison of airway inflammation scores and total PMN cell counts in upper and lower airways, average scores for both airway structures were calculated.

Results

Structural Findings

Clinical acute rhinosinusitis was evident in all groups with hyperemic, congested and vascularized sinonasal mucosae and purulent secretion in some of the sinuses. There was no evidence of obvious inflammation or hemorrhage in trachea or lung tissues (using a Zeiss-Opmi 99 surgical microscope).

Table 1 Scoring system for airway inflammation

Score	Description
0	Normal, epithelium intact, no PMN cell evident
0.5	Engorgement of blood vessels, hyperemia, PMN cells in capillaries
1	PMN cells evident in perivascular tissue as well as in capillaries
2	PMN cells located within the intact epithelium; few PMN cells within the muscular or adventitial layers
3	Large numbers of PMN cells within the epithelium and, to a lesser extent, in the airway lumen; epithelium remains mostly intact
4	Masses of PMN cells infiltrating the entire wall down to and including the adventitia; the epithelium is disrupted; at least 50% of the airway circumference involved

Histological Results

Haematoxylin and eosin stain of the sham control sections with a magnification of $\times 100$ were shown in Fig. 1. There was no evidence of inflammation in any section of the airways, showing normal pseudostratified ciliated columnar epithelium of the nasal mucosa (Fig. 1a) and the trachea (Fig. 1b), and alveolar epithelium (Fig. 1c). Glands and collagen fibers are seen at subepithelial area.

Histological evaluation of the mucosal specimens of study group with hematoxylin and eosin stain showed consistent inflammation in nearly all sections. Examination with magnifications of $\times 40$ and $\times 400$ revealed a progressive course of acute inflammatory changes varying from engorgement of vessels and PMN cell proliferation within the capillaries, in the perivascular tissue of the epithelium or in the lamina propria and to epithelial disruption. Increased hypercellularity and PMN cell infiltration surrounding terminal bronchioles were observed on histological evaluation of the lung sections.

The minimum inflammation score was 0.7 which means PMN evident in capillaries and perivascular tissue in some extent. Examples of some histological scoring level were shown in Figs. 2 and 3 for the upper airway and in Figs. 4 and 5 for the lower airway respectively. The average airway inflammation scores were 2.86 ± 1.81 for upper airway structures (right ethmoid sinus, right maxillary sinus, left ethmoid sinus, left maxillary sinus and sphenoid sinus) and 1.36 ± 0.77 for lower airway structures (upper trachea, lower trachea, right mainstem bronchus, right proximal airway, right distal airway, left mainstem bronchus, left proximal airway and left distal airway), respectively. The difference between the two airways was significant ($P < 0.01$). Average airway inflammation scores in upper and lower airway structures of each animal are shown in Fig. 6.

All airway sections in the study group showed PMN cell infiltration more or less. The average PMN cell counts were 33.3 ± 2.96 for upper airway structures and 11.1 ± 2.51 for lower airway structures, respectively (Fig. 7). The difference between the two airways for PMN cell count was

significant ($P < 0.01$). The smallest average PMN cell count was at lower airway sections of one of the subjects, which was 8.4.

Discussion

The upper airway, specifically the nasal epithelium, resembles lower airway in many features histologically and functionally. The structure of the nasal mucosa is similar to that of the bronchial mucosa. Both mucosae are characterized by the presence of a ciliated pseudostratified columnar epithelium, and both submucosae contain mucous glands, blood vessels, connective tissue, nerves, and inflammatory cells. Upper and lower airways are also similar from functional point of view. The upper respiratory tract functions as a physical filter, resonator, heat exchanger, and humidifier of exchanged air. Failure of any of these functions clearly alters the homeostasis of the lower respiratory tract [1, 2].

Although the histological and clinical association of allergic rhinitis and bronchial asthma are rather well-defined, there is little definitive information as to whether rhinosinusitis contributes to the pathogenesis of bronchiolitis or other lower respiratory tract infections. There are studies concerning measurement of nasal inflammatory markers showing that it can generally be assumed to reflect lower airway pathology [5, 11–15]. Jyonouchi et al. [5], have evaluated distribution of IgG antibodies in the airway in association with inflammatory changes in a rabbit sinusitis model. They measured IgG antibody and lactate dehydrogenase levels in solutions from sinus, lower airway and middle ear lavage and in serum at the first, second, third and fourth weeks following inoculation of maxillary sinuses of rabbits with *Bacteroides fragilis*. It has been found that IgG antibody levels in the upper airway mucosa increase within two weeks following bacterial inoculation as a part of mucosal immune responses. IgG antibodies were detected in most bronchoalveolar lavage samples after two weeks. The response of the lower airway to the exposure of the upper airway to allergens, viral infection, and to different exogenous harmful substances, such as

Fig. 1 Sham control sections. **a** Nasal mucosa. Normal pseudostratified ciliated columnar epithelium of maxillary sinus, *E* Epithelium, *G* glands, *C* Collagen fibers. **b** Trachea. *E* Epithelium, *Lp* Lamina propria, *HC* Hyalin Cartilage. **c** Lung tissue. *A* Alveoli, *T* Terminal bronchiole, *arrows* Alveolar epithelium. Hematoxylin & Eosin (H & E), Magnification (M) $\times 100$

ozone, passive smoking, or particles have been studied. In exposure studies with ozone [14] or swine dust in adults [15], changes in nasal lavage samples reflect, to some extent, inflammation in the lower airway. In experimentally induced infection with rhinovirus, it has been shown that the virus can be found both in the upper and lower airway [12]. In wheezy infants, an increase in tumor necrosis

Fig. 2 Ethmoid sinus mucosa of the subject 2. Large numbers of PMN cell within the capillaries. *E* Epithelial cells, *C* Capillary, *arrows* PMN cells. H & E, $\times 400$ OM

Fig. 3 Maxillary sinus mucosa of the subject 4. Masses of PMN cell infiltrating the entire wall down to and including the adventitia; the epithelium is disrupted. *Right pointing black triangle* PMN cells, *F* Fibroblast proliferation, *arrows* Epithelial disruption. H&E, $\times 400$ OM

factor a concentrations in nasal lavage samples has been observed, particularly in children infected with the respiratory syncytial virus [13]. These data suggest that local inflammation, which promotes lower airway inflammation, can be showed and correlated by the nasal inflammation markers. In this article, we addressed the extent to which nasal bacterial infection-induced inflammation can provide information on lower airway inflammation histologically.

On another perspective, to show the effects of bacterial inflammation of lung on upper airways, suggesting the concept of ‘one airway—one disease’ has been studied in some previous studies: Roberts et al. [16] showed high

Fig. 4 Upper tracheal section of the subject 2. PMN cell evident in perivascular tissue and capillaries. *E* Epithelial cells, *C* Capillary, arrows PMN. H&E, $\times 400$ OM

Fig. 5 Lower tracheal section of the subject 2. PMN cell infiltration all through the terminal bronchioles and alveoli. *T* Terminal bronchiole, *A* Alveoli, arrows PMN cells. H&E, $\times 400$ OM

prevalence of chronic nasal symptoms in patients with chronic obstructive pulmonary disease and have gone on to show that these contribute to impairment in quality of life. It was shown that, 75% of patients with moderate-to-severe chronic obstructive pulmonary disease experienced persistent daily nasal symptoms, most commonly rhinorrhea. More recently, an analysis [17] of matched nasal and bronchial biopsy specimens has suggested that nasal and bronchial inflammation coexist in chronic obstructive pulmonary disease patients. Lower airway inflammation in chronic obstructive pulmonary disease patients is known to be modulated by bacterial colonization [18]. Nasal inflammation has been shown in patients with chronic

Fig. 6 Airway inflammation scores in upper and lower airway structures of each animal. Average scores for upper airway structures (right ethmoid sinus, right maxillary sinus, left ethmoid sinus, left maxillary sinus and sphenoid sinus) and lower airway structures (upper trachea, lower trachea, right mainstem bronchus, right proximal airway, right distal airway, left mainstem bronchus, left proximal airway and left distal airway) were calculated for each subject

Fig. 7 Total polymorphonuclear cell counts in upper and lower airway structures of each animal. Average scores for upper airway structures (right ethmoid sinus, right maxillary sinus, left ethmoid sinus, left maxillary sinus and sphenoid sinus) and lower airway structures (upper trachea, lower trachea, right mainstem bronchus, right proximal airway, right distal airway, left mainstem bronchus, left proximal airway and left distal airway) were calculated for each subject

obstructive pulmonary diseases. High prevalence of chronic nasal symptoms has been shown in these patients [6, 8]. Vachier et al. [18] has also suggested the presence of neutrophilic inflammation in the nasal airways of patients with chronic obstructive pulmonary disease. Hurst et al. [17] have demonstrated increased levels of the neutrophil chemoattractant protein IL in the upper airway of patients when compared to control subjects. The upper airway IL-concentration correlated with that in the lower airway,

and at both sites the concentration was related to indexes of bacterial colonization. They suggested correlation between the degree of upper and lower airway inflammation in chronic obstructive pulmonary disease patients [6].

We used rabbits as an experimental model, because they have practical advantages and their respiratory tract structures have anatomical and pathological conditions similar to humans. Rabbits are the most commonly used animals for investigation of sinus and lower airway diseases in the literature [4, 5, 9, 10, 19]. In order to induce acute rhinosinusitis, 0.05 ml of solution containing 2×10^7 CFU of *Staphylococcus aureus* soaked in absorbable gelatin sponge (Spongostan, Johnson & Johnson Medical Limited, Gargrave, Skipton, UK) was inoculated into the nasal cavities of animals as described early in Marks et al's study [9]. *Bacteroides fragilis* have also been used for inoculation of maxillary sinuses of rabbits in some similar studies [5, 19].

This model produced a marked sinus infection and a relatively less marked lower respiratory tract infection that were characterized by epithelial and subepithelial infiltration with PMN cells, and epithelial degeneration. In contrast, the sham control subject showed no any signs of inflammation.

It has been shown that nasal inflammation is present in asthmatic patients with or without nasal symptoms, but airway remodeling seems to be less extensive in the nasal mucosa than in the bronchial mucosa. Patients with asthma have a thicker basement membrane, smooth muscle hypertrophy, and greater epithelial desquamation, whereas the nasal epithelium of those with rhinitis alone is less damaged [2]. These pathophysiologic similarities between the asthma and allergic rhinitis have resulted in the concept of an inflammatory “cross-talk” between the nose and the lung. Our results suggest that in subjects with nasal bacterial inflammation there is also pan-airway inflammatory response. Higher inflammation scores in upper airways have suggested to be reflecting the primary site of infection. Our findings of similar inflammatory process in the upper and lower airways suggest that the nose deserves further study and that it may provide more accessible site for future lower airway inflammatory disease research.

A number of mechanisms have been suggested to explain the link between the upper and lower airway, including the direct passage of mediators along the respiratory mucosa, blood-borne passage, and neural responses [3, 4, 8, 20–23]. Our finding of higher nasal inflammation scores suggests possible mechanism for the relationship between upper and lower airway inflammation is passing of bacteria into the lower respiratory tract via direct colonization. Brugman et al. [10] have demonstrated the relationship between postnasal drip and the presence of lower airway inflammation in bacterial rhinosinusitis induced

rabbits. Another mechanism that might link an inflammatory process in the upper respiratory tract to a change in lower airways function is the elicitation of a nasobronchial reflex [10]. Complex interactions among several of the potential mechanisms may influence the ultimate relationship between rhinosinusitis and lower airway dysfunction.

Key Messages

- This study clearly demonstrates that, whatever the mechanism that might link an inflammatory process in the upper respiratory tract to a change in lower airways function is, there is an interrelation between the both airways histopathologically.
- We obtained a generalized mucosal inflammatory response against localized bacterial inflammation in a rabbit model of acute rhinosinusitis, confirming the suggestion of ‘one airway—one disease’.

Conflict of interest None.

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