Effect of Smoking Cessation on the Microbial Flora

Itzhak Brook, MD, MSc; Alan E. Gober, MD

Objective: To determine the effect of smoking cessation on the frequency of recovery of potential pathogens and aerobic and anaerobic interfering bacteria in the nasopharynges of smokers.

Design: Nasopharyngeal cultures were taken from 20 smokers before and 12 to 15 months after cessation of smoking. Potential pathogens and aerobic and anaerobic bacteria with interfering capabilities against these organisms were identified.

Results: Eleven potential pathogens (0.92 pathogens per subject) were isolated from nasopharyngeal cultures obtained from 9 individuals before smoking cessation, and 2 (0.17 per subject) were recovered from 2 individuals after smoking cessation ($P<0.05$). Bacterial interference between 2 aerobic (α and nonhemolytic streptococci) and 2 anaerobic species (Prevotella and Peptostreptococcus species) and 4 potential pathogens (Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pyogenes) was observed. Bacterial interference was noted in 35 instances against the 4 potential pathogens by 14 normal flora isolates that were recovered from the smokers before cessation and in 116 instances by 42 isolates after cessation ($P<0.01$).

Conclusion: To our knowledge, these findings illustrate for the first time that the high number of pathogens and low number of interfering organisms found in the nasopharynx of smokers revert to normal levels after complete cessation of smoking.


Smoking is associated with an increased risk of respiratory tract infection in adults and also with oral colonization by some potentially pathogenic microorganisms. In children, exposure to cigarette smoke is a risk factor for respiratory tract infection and meningococcal meningitis. Active smoking and passive exposure to cigarette smoke is also associated with carriage of potentially pathogenic species of bacteria in both adults and children possibly owing to enhanced bacterial binding to epithelial cells of smokers and the low number of α-hemolytic streptococci with inhibitory activity against Streptococcus pyogenes in the oral cavity of smokers. The nasopharyngeal flora of smokers contain fewer aerobic and anaerobic organisms with interfering capability and more potential pathogens compared with those of nonsmokers.

The purpose of this study was to determine the effect of smoking cessation on the frequency of recovery of potential pathogens and aerobic and anaerobic interfering bacteria in the nasopharynx of smokers.

Methods

Patients

Twenty healthy adults who had smoked at least 10 cigarettes a day for the past 5 years and had completely ceased smoking were included in the study. None were immune deficient or had any chronic illness such as otitis, sinusitis, or tonsillitis; had received antimicrobial therapy; or had had a respiratory tract infection in the past 3 months prior to the time when the first and second culture samples were taken. The mean age of the patients was 34 years (range, 21-52 years), and 8 were men.

Two culture samples were obtained from each individual: 1 sample before cessation of smoking and the other 12 to 15 months after they stopped smoking. The culture specimens were taken using sterile calcium alginate swabs and were collected from the retropharynx (through the mouth), and were...
immediately plated into media supportive of the growth of aerobic and anaerobic bacteria. The protocol was approved by the institutional review board.

MICROBIOLOGIC FINDINGS

Sheep’s blood (5%), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The culture plates were incubated aerobically at 37°C (MacConkey agar) and under 5% carbon dioxide (blood and chocolate agars), and they were examined at 24 and 48 hours. For the recovery of anaerobic bacteria, the specimens were inoculated onto pre-reduced vitamin K₁–enriched Brucella blood agar, blood agar that contained kanamycin sulfate and vancomycin, and an aerobic blood plate that contained phenylethyl alcohol and enriched thioglycolate broth. These media were immediately inoculated in anaerobic containers (Gas Pack; Baltimore Biological Laboratories, Cockeysville, Md) at 37°C and examined after 48 and 96 hours of incubation at 37°C. All types of colonies on each plate were isolated. The mean total number of aerobic and anaerobic bacterial isolates processed from each individual was 7.8 (range, 4-17). Aerobic and anaerobic bacteria were identified by previously described methods.¹⁰

TESTING FOR INTERFERENCE

The inhibitory activity was tested in a blind fashion, against 1 strain each of a recent clinical isolate of Streptococcus pneumoniae, Haemophilus influenzae (non–type b), Moraxella catarrhalis, and S pyogenes. Inhibitory activity of 5 separate colonies of all aerobic and anaerobic isolates was evaluated. The inhibitory activity of each isolate was individually tested against the test organisms, using the Steer steel pin replicator as previously described.¹¹ In brief, minidrops of log-phase broth cultures of the isolates were transferred with the pin replicator to vitamin K₁–enriched Brucella blood or chocolate (for H influenzae) agar plates and allowed to dry for 15 minutes at room temperature. A sample of a log-phase broth culture of the target strain was applied adjacent to each of the isolated strains, and the plates were incubated in 5% carbon dioxide or anaerobically at 37°C for 48 hours. Bacterial interference was considered present when inhibition of growth of the target strain was reproducibly detected adjacent to the isolated strains. Degrees of inhibition varied from complete absence of growth to a narrow zone of poor growth along the proximal area of the colony.

Statistical analysis was done using the Fisher exact test. The P values are 2 sided.

RESULTS

Eleven potential pathogens (0.92 per subject) were isolated from nasopharyngeal cultures obtained from 9 of the 20 individuals before smoking cessation, and 2 (0.17 per subject) were recovered from 2 individuals after smoking cessation (P<.05) (Table 1). One of these individuals was colonized with the same pathogen (H influenzae) prior to cessation of smoking. Bacterial interference between 2 aerobic (α-streptococci and nonhemolytic streptococci) and 2 anaerobic species (Prevotella and Peptostreptococcus species) and 4 potential pathogens (S pneumoniae, H influenzae, M catarrhalis, and S pyogenes) was observed. Bacterial interference was noted in 35 instances against the 4 potential pathogens by 14 normal flora isolates that were recovered from the smokers before cessation, and in 116 instances by 42 isolates after cessation (P<.01) (Table 2).

COMMENT

This study compares the recovery rate of potential pathogens as well as interfering aerobic and anaerobic bacteria from the nasopharynges of smokers before cessation of smoking and 12 to 15 months after cessation of smoking. Our findings confirm those from our previous study⁹ that the nasopharyngeal flora of smokers contain fewer aerobic and anaerobic organisms with interfering capability and more potential pathogens compared with non-smokers. However, to our knowledge, we have observed for the first time that the high number of pathogens

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### Table 1. Number of Isolates of Pathogens in the Nasopharynges of 12 Smokers Before and After Smoking Cessation

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Before Smoking Cessation</th>
<th>After Smoking Cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Haemophilus influenzae (non–type b)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. Number of Isolates With Interfering Capabilities Recovered in the Nasopharynges in 12 Smokers Before and After Smoking Cessation

<table>
<thead>
<tr>
<th>Target Organisms</th>
<th>Before Smoking Cessation</th>
<th>After Smoking Cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP</td>
<td>HI</td>
</tr>
<tr>
<td>α-Hemolytic streptococci</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nonhemolytic streptococci</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Prevotella species</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Peptostreptococcus species</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: GAS, group A β-hemolytic streptococci (Streptococcus pyogenes); HI, Haemophilus influenzae; MC, Moraxella catarrhalis; SP, Streptococcus pneumoniae.

*Ratio of total instances of bacterial interference to number of interfering isolates: 35:14 before smoking cessation, 116:42 after smoking cessation.
and low number of interfering organisms revert to normal levels after complete cessation of smoking. These levels were similar to those we had observed in nonsmokers in a previous study.9  

Haemophilus influenzae, S pneumoniae, and M catarrhalis were more frequently isolated in subjects who were prone to sinusitis24 and otitis media,1.14 and S pyogenes was more often isolated in children prone to tonsillitis15 than in those not prone to infections. Conversely, aerobic and anaerobic interfering organisms were more often recovered in those patients who were not prone to infection compared with those who were prone to upper respiratory tract infections.12-15 Smoking was a risk factor in patients with chronic obstructive pulmonary disease for colonization with Pseudomonas aeruginosa,10 H influenzae,16.17 S pneumoniae, and M catarrhalis.17  

The presence of organisms with interfering potential may play a role in the prevention of upper respiratory tract infections. Their absence in smokers may contribute to their increased risk of acquiring respiratory pathogens and their greater susceptibility to respiratory infections.1 The enhanced binding of pathogenic bacteria to epithelial cells of smokers may account for their predominance.7 It is also possible that the prior frequent use of antibiotics in smokers may have reduced the number of organisms inhibitory to the growth of pathogens. However, we examined only individuals who had not received antibiotic drugs in the past 3 months; prior antibiotic use does not explain the lack of such organisms in these individuals.  

The ability of the indigenous normal nasopharyngeal flora to inhibit colonization with potential pathogens has been reported in several studies of subjects with upper respiratory tract infections.16-22 α-Hemolytic streptococci were found to inhibit the colonization in patients and in vitro growth of a variety of pathogenic bacteria. These include S pneumoniae, S pyogenes, and S aureus.16-22 The production of bacteriocin and other inhibitory substances that suppress some bacterial growth, or utilization of nutrients in the nasopharyngeal environment essential for the potential pathogens, may explain this phenomenon.23  

Therapeutic colonization of the nasopharynx with interfering bacteria was recently studied by Roos et al,24 who attempted to colonize children with recurrent tonsillitis with α-hemolytic streptococci or placebo. Clinical recurrences occurred in 1 (2%) of 51 children in the α-hemolytic streptococci group and 14 (23%) of 61 children in the placebo-treated group. Similarly, these investigators showed that colonization with α-hemolytic streptococci with the ability to inhibit the growth of pathogens (“interfering” activity) reduced the recurrence of acute otitis media and the frequency of otitis media with effusion in susceptible children.25 Three months after colonization with α-hemolytic streptococci, 22 (42%) of the children given the streptococcal spray were free of otitis media and had a healthy tympanic membrane compared with 12 (22%) of those given placebo.  

In conclusion, our study demonstrates the beneficial effects of smoking cessation in restoring the interfering aerobic and anaerobic bacteria, which are potentially beneficial bacteria that can interfere with the growth of potential pathogens. The possible role of interfering bacteria and the potential recolonization with α-hemolytic streptococci has yet to be studied in patients with chronic obstructive pulmonary disease who smoke. Further studies of smokers are warranted to investigate whether colonization of the nasopharynx with interfering organisms and/or cessation of smoking would be beneficial, allowing for the return of the normal inhibitory flora and the reduction in the number of pathogens.

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Correspondence: Itzhak Brook, MD, MSc, 4431 Albe-marle St NW, Washington, DC 20016 (IB6@georgetown.edu).  

Author Contributions: Drs Brook and Gober had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Brook. Acquisition of data: Brook and Gober. Analysis and interpretation of data: Brook. Drafting of the manuscript: Brook and Gober. Critical revision of the manuscript for important intellectual content: Brook. Administrative, technical, and material support: Brook and Gober. Study supervision: Brook.  

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