Rhinovirus RNA in the Maxillary Sinus Epithelium of Adult Patients with Acute Sinusitis

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We used in situ hybridization for the detection of rhinovirus in maxillary sinus biopsy specimens obtained from 14 adult patients with acute sinusitis. In 7 specimens, rhinovirus RNA could be demonstrated in the maxillary sinus epithelium, thereby confirming the etiology of rhinovirus and the clinical suspicion of acute sinusitis.

Most cases of acute sinusitis are considered to result from a previous respiratory viral infection, for which rhinoviruses are the most common cause. However, the role and relative importance of viral infections are far from clear. Previous studies have indicated that rhinovirus infections can be associated with maxillary sinusitis [1–3], but it is uncertain how often active viral replication occurs in the maxillary sinus epithelium, if it occurs at all. The presence of the virus in the maxillary sinus may also be the result of the virus being replicated in some place other than the upper respiratory tract epithelium and then being introduced into the maxillary sinus during nose blowing [4]. An important question with regard to understanding the pathogenesis of sinusitis is whether viral invasion of the sinus cavity occurs or whether inflammatory events in the osteomeatal area are responsible for the development of sinusitis. The objective of the present study was to determine, by in situ hybridization, whether rhinovirus RNA can be detected in the maxillary sinus epithelium of adult patients with acute sinusitis.

Nine adult patients (8 women [age, 21–43 years] and 1 man [age, 40 years]) with diagnoses of acute community-acquired sinusitis were studied from January 1998 through May 1998 at the Department of Otorhinolaryngology of the University Hospital of Helsinki. For each case, diagnosis was based on clinical findings. All patients had been admitted to the clinic to undergo maxillary sinus puncture for clinical sinusitis and/or worsening of sinusitis symptoms. Symptoms noted at the time of admission were as follows: nasal obstruction, purulent rhinorrhea, facial pain, and/or fever. One patient had been given a diagnosis of left lobar pneumonia 1 week prior to admission, and another patient had otitis media diagnosed 3 days prior to admission; for both of these patients, antibiotic treatment had been initiated during the period of primary care. For all patients, the duration of sinusitis symptoms was ≤2 weeks. Patients for whom chronic sinusitis and/or recurrent sinusitis problems had been suspected were excluded from the study, as were patients who had a history of allergic rhinitis or asthma. In addition, 5 male conscripts (age, 21–23 years) from the Department of Ear, Nose and Throat of the Central Military Hospital in Helsinki were studied and underwent maxillary sinus puncture for acute maxillary sinusitis in March 1999.

According to radiography findings, a total of 12 patients had sinusitis-related changes (either a change in the air-fluid level or total opacity) in their maxillary sinuses. Radiography of the sinus was not performed for 2 of the patients. All patients were treated (or continued receiving treatment) with an oral antibiotic (usually amoxicillin) for 10 days. A follow-up visit was scheduled 1 week after the start of treatment; thereafter, visits occurred once weekly if symptoms persisted. One maxillary sinus biopsy specimen was obtained when the patient with exophthalmus due to Morbus Basedow disease underwent orbital decompression surgery, and this specimen served as a control for noninfectious maxillary sinus epithelium.

The special needle was passed into the sinus cavity under the lower turbinate, and a tiny forceps was passed through the needle. Only one maxillary sinus biopsy specimen was collected per patient. Each specimen was placed into formalin and then was embedded in paraffin, cut, and fixed on the microscope slides. After collection of the biopsy specimens, antral lavage was performed for every patient.

The genetic region representing part (nucleotides 332–572) of the rhinovirus type 14 (rhinovirus-14) 5′-untranslated region (5′ UTR) was amplified, by PCR, from a complementary DNA template [5] and was cloned into pGEM-4Z plasmid (Promega) between the HindIII and XbaI sites. The 240-nucleotide fragment
Figure 1. In situ hybridization of HeLa cells infected with rhinovirus type 14 (rhinovirus-14). HeLa cells infected with rhinovirus-14 (A) or uninfected control cells (B) were harvested after 24 h, were fixed, and were embedded in paraffin. In situ hybridization was performed with rhinovirus-14 antisense probe. In panel A, positive signal is seen as a cytoplasmic brown color. Bar = 5 μm.

Figure 2. In situ hybridization for rhinovirus type 14 (rhinovirus-14) in maxillary sinus biopsy specimens. A, Detection of rhinovirus-14, antisense probe, in a maxillary sinus biopsy specimen obtained from the patient with maxillary sinusitis. Positive hybridization signal is visible as cytoplasmic brown reactivity in respiratory epithelial cells. B, Adjacent section from the same biopsy specimen, probed with rhinovirus-14 sense probe, demonstrates no hybridization signal. C, Rhinovirus-14 antisense-negative maxillary sinus biopsy specimen obtained from another patient with acute sinusitis. D, Lack of rhinovirus-14 reactivity in a maxillary sinus biopsy specimen obtained from the maxillary sinus of an uninfected patient during decompression surgery. Bar = 10 μm.

was cloned in both orientations under the control of T7 promoter. Digoxigenin-labeled RNA probes were synthesized using T7 RNA polymerase and the DIG RNA labeling kit (Roche).

Five-millimeter-thick sections for in situ hybridization were mounted in ribonuclease-free conditions on heat-treated Super Frost (+) slides (Menzel-Gläser). In situ hybridization was performed using the automated Gen II slide stainer (Ventana Medical Systems) for in situ hybridization/immunohistochemistry. The program used was the "Ventana Regular" protocol. Sections were first deparaffinized in 3 changes of xylene for 15 min and then were hydrated for 3 min in 2 changes of absolute 96% and 70% ethanol. After they were rinsed in distilled water treated with 0.1% diethylpyrocarbonate, the slides were kept in alkaline phosphatase solution (Ventana Medical Systems). The sections were treated with Protease 3 Reagent (Ventana Medical Systems) for 8 min before hybridization was done at 45°C for 14–15 h. The hybridization mixture contained 50% formamide and ×5 standard saline citrate (SSC). After hybridization, 3 washes were performed at 65°C for 8 min in 1 × SSC, 0.5 × SSC, and 0.1 × SSC. Monoclonal antidigoxigenin antibody (Clone DI-22; Sigma) was incubated with the sections for 28 min. The probe was detected with the 3,3′-diaminobenzidine tetrahydrochloride biotin avidin detection kit (Basic DAB; Ventana Medical Systems). The sections were dehydrated and mounted with coverslips with Eukitt (O. Kindler, GmbH), and they were analyzed by use of an Olympus light microscope.

In the beginning, the rhinovirus-14 probes were tested in both rhinovirus-14–infected and rhinovirus-14–uninfected HeLa cell cultures in vitro. Figure 1 shows examples of rhinovirus-14–infected and rhinovirus-14–uninfected HeLa cells. Positive signals were found in the cytoplasm of infected HeLa cells by use of the antisense probe (figure 1A), but they were not detected by use of the sense probe (data not shown). Uninfected HeLa cells tested negative for rhinovirus-14 by use of the antisense probe (figure 1B) and the sense probe (data not shown).

By in situ hybridization with the antisense probe (figure 2A)—but not with the sense probe (figure 2B)—rhinovirus RNA was detected inside the respiratory epithelium cells in 7 (50%) of 14 maxillary sinus biopsy specimens obtained from patients with acute sinusitis. No signal was observed in maxillary sinus biopsy specimens obtained from a patient without
maxillary sinusitis (figure 2D). Biopsy specimens from all 14 patients who were treated for sinusitis demonstrated histopathological features of acute infection. Some epithelial degeneration and increased mucus formation were evident, and an increased mucosal inflammatory cell infiltration was noted (figure 2C). Inflammatory changes were not seen in the control patient who underwent surgery for Morbus Basedow disease (figure 2D).

In this study, we have shown that rhinovirus RNA can be found inside the epithelial cells of the maxillary sinus in 50% of samples obtained from patients with acute sinusitis, by use of in situ hybridization with rhinovirus-14 probe. Epidemiological studies have shown that rhinovirus shedding in nasal fluid persists for up to 3 weeks in patients with colds due to rhinovirus [6]. Unfortunately, we did not obtain convalescent-phase maxillary sinus biopsy specimens from our patients; however, in a recent experimental study of the lower respiratory tract, it was shown that 2 of 5 bronchial biopsy specimens remained positive for rhinovirus type 16 in situ for 6–8 weeks after experimentally induced rhinovirus infection [7]. The possibility that, for some of our patients, detection of rhinovirus-14 would represent a previous infection, rather than an acute infection, cannot be ruled out; however, patients with chronic sinusitis and patients with recurrent sinusitis problems were not included in our study.

On the other hand, it is possible that a combination of rhinovirus probes covering several serotypes could have increased the sensitivity of the rhinovirus in situ hybridization protocol. Human rhinoviruses are known to consist of ≥2 genetic clusters that also seem to be found in the 5′ UTR [8, 9]. In this study, no testing was done to determine how broadly the rhinovirus-14 probe reacted with other rhinoviruses that belong to the same genetic cluster. Our finding that a large number of sinus epithelial cells were positive for rhinovirus is in contrast to findings of an earlier study [10], in which only a small number of nasal epithelium cells were determined to be positive for rhinovirus by in situ hybridization. It remains unknown whether this is a result of individual variation, an epidemiological situation, or the differences between clinical sinusitis and experimental rhinovirus infection. We conclude that direct demonstration of rhinovirus RNA in situ provides evidence of the presence of rhinovirus in the respiratory epithelial cells in the maxillary sinus of adult patients with acute sinusitis.

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References