from patient throat swabs. Furthermore, the effects of neuraminidase inhibitors on the release of influenza virus from human tracheal epithelial cells were examined to establish a new strategy for evaluating the effect of neuraminidase inhibitors on influenza viral replication in human airway epithelial cells. Four types of inhibitors reduced oseltamivir-sensitive influenza A/H1 pdm 2009 viral titers in supernatants and viral RNA in cells. In contrast, oseltamivir did not reduce viral titers and viral RNA of the oseltamivir-resistant seasonal influenza A/H1N1 virus. These findings suggest that accurate information related to the effectiveness of neuraminidase inhibitors on influenza virus replication in human airways can be acquired rapidly using the methods reported in this study. This information will be useful for physicians during the treatment of patients with influenza virus infection.

IL-6 and TNF-α are associated with airway inflammation during seasonal influenza viral infection [Hayden et al., 1998] and with the induction of apoptosis in cells [Ruwanpura et al., 2011; Gao et al., 2012]. The results of the present study demonstrated that four types of neuraminidase inhibitors can reduce the concentrations of IL-6 and TNF-α in supernatants after influenza A/H1 pdm 2009 viral infection, but treatment with oseltamivir does not reduce the concentrations of these cytokines after oseltamivir-resistant seasonal influenza A/H1N1 viral infection. Therefore, the inflammatory cytokines that are produced in tracheal epithelial cells may be related to airway damage induced by infection with the influenza A/H1 pdm 2009 virus and the seasonal influenza A/H1N1 virus. Furthermore, the methods established in the present study can be used to measure not only the viral titers but also the levels of inflammatory cytokines in the supernatants.

Affinity for and growth within the epithelium varies among different strains of influenza viruses. In fact, the maximum viral titers in the supernatants of cells infected with the influenza A/H1 2009 virus and the oseltamivir-resistant seasonal influenza A/H1N1 virus in this study were higher than the previously reported titers in the supernatants of cells infected with the seasonal influenza H3N2 virus [Yamaya et al., 2010]. The viral titers in the lungs of mice were also different among the strains [Kubo et al., 2010]. Kubo et al. [2010] determined the area under the curves (AUCs) of the time course changes of viral titers in the lungs of mice treated with zanamivir and laninamivir and calculated the ratio, which was compared to the AUC in mice treated with saline, to examine the effectiveness of the drugs against influenza virus infection. In methods that use cultured cells, the AUC ratio may indicate the magnitude of the effect of neuraminidase inhibitors on viral release. The IC_{90} of neuraminidase inhibitors for viral titers in the supernatants of the cells 24 hr after infection has also been reported [Itoh et al., 2009]. These parameters may standardize the observed differences in affinity for and growth in the epithelium among influenza strains.

In summary, the treatment of primary cultures of human tracheal epithelial cells with the neuraminidase inhibitors oseltamivir, zanamivir, laninamivir, and peramivir reduced the viral titers of influenza A/H1 pdm 2009 in cell supernatants in the present study. In contrast, oseltamivir did not reduce the titers of the oseltamivir-resistant seasonal influenza A/H1N1 virus. Because the virus stocks were prepared by infecting human tracheal epithelial cells using nasal swabs, these infection methods may provide accurate and rapid information related to the effects of neuraminidase inhibitors on influenza viruses that are isolated clinically from patients.

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