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Oseltamivir Inhibits H7 Influenza Virus Replication in Mice Inoculated by the Ocular Route

Jessica A. Belser, Katrina Sleeman, Melissa B. Pearce, Jacqueline M. Katz, Larisa V. Gubareva, and Terrence M. Tumpey

Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The majority of human infections associated with H7 influenza viruses have resulted in ocular and not respiratory disease. While oseltamivir has been prescribed to individuals presenting with conjunctivitis following H7 virus exposure, it is unknown if oseltamivir inhibits virus replication in ocular tissue. We demonstrate that H7 viruses possess sensitivity to neuraminidase inhibitors and that administration of oseltamivir before ocular virus challenge in mice inhibits H7N7 and H7N3 virus replication in ocular and respiratory tissues.

Neuraminidase (NA) inhibitors are the most widely used class of antiviral drug for influenza viruses and are well documented to alleviate the symptoms of respiratory disease following seasonal influenza virus infection in humans (11). However, unlike other influenza virus subtypes that cause predominant respiratory disease in humans, H7 influenza viruses frequently result in ocular rather than respiratory symptoms (3). Oseltamivir prophylaxis has nonetheless been prescribed during several H7 virus outbreaks resulting in human infection, including H7N7 virus in The Netherlands in 2003, H7N3 virus in Canada in 2004, and H7N2 in Wales in 2007 (9, 16, 25) (Table 1). Retrospective studies have examined the role of oseltamivir prophylaxis in developing conjunctivitis and/or influenza-like illness following H7 virus exposure, finding that the prophylactic use of oseltamivir resulted in a decreased risk of virus infection, including self-reported conjunctivitis (16, 17, 20, 22). While a limited number of studies have demonstrated the sensitivity of H7 viruses to currently available antiviral drugs (13, 14, 16, 21), the ability of oseltamivir to inhibit H7 virus infection has not been examined *in vivo*. Furthermore, it has not been shown experimentally if prophylaxis with neuraminidase inhibitors can reduce directly the presence of virus in the eye or inhibit virus spread from ocular sites to respiratory tract tissues. Previous studies from our laboratory have identified that intranasal and ocular inoculation of virus results in different kinetics of virus replication and spread *in vivo*, underscoring the need to examine the efficacy of antiviral treatments for multiple routes of exposure (5; J. A. Belser, K. M. Gustin, T. R. Maines, J. M. Katz, and T. M. Tumpey, unpublished data).

While previous work has identified strain-specific differences in NA inhibitor sensitivity within virus subtypes (27), only a few H7 subtype viruses have been evaluated to date. Numerous differences have been identified between the Eurasian and North American lineages of H7 influenza viruses, including their virulence in mammals, hemagglutinin receptor-binding preference, and induction of host innate immune responses (1, 2, 4, 6, 15). To determine the sensitivity to existing NA inhibitors of H7 viruses of both lineages associated with disease in humans, we performed an NA activity inhibition assay with the NA inhibitors oseltamivir carboxylate (Roche), zanamivir (GlaxoSmithKline), and perami-

TABLE 1 Sensitivity of H7 influenza viruses associated with disease in humans in the NA inhibition assay with MUNANA[d] substrate

Virus[a]	Subtype[b]	Patient symptom(s)[a]	Mean $IC_{50} \pm SD$ (nM)[c]		
			Zanamivir	Oseltamivir carboxylate	Peramivir
A/Netherlands/219/2003	HPAI H7N7	Respiratory, fatal	6.72 ± 0.97	3.28 ± 0.37	1.54 ± 0.01
A/Netherlands/230/2003	HPAI H7N7	Conjunctivitis	3.36 ± 0.42	1.05 ± 0.05	0.62 ± 0.09
A/Canada/504/2004	HPAI H7N3	Conjunctivitis	2.95 ± 0.16	1.64 ± 0.22	1.32 ± 0.15
A/Canada/444/2004	LPAI H7N3	Conjunctivitis	3.41 ± 0.13	2.05 ± 0.11	1.56 ± 0.04
A/New York/107/2003	LPAI H7N2	Respiratory	2.58 ± 0.33	0.55 ± 0.06	0.76 ± 0.11

[a] Source information for clinical isolates and patient information was published previously (3, 10, 25). Identical virus stocks were used for *in vitro* and *in vivo* experiments.
[b] HPAI, highly pathogenic avian influenza virus; LPAI, low-pathogenicity avian influenza virus.
[c] Mean IC_{50} ± standard deviation (SD) values were calculated from data collected from duplicate independent experiments.
[d] MUNANA, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid.

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Address correspondence to Terrence M. Tumpey, tft9@cdc.gov.

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TABLE 2 Effect of orally administered oseltamivir on virus titers in mice inoculated by the ocular route with H7 influenza viruses

Tissue	Day p.i.	No. of mice with virus detected/total[a]			
		NL/219		Can/504	
		Oseltamivir[b]	No treatment[c]	Oseltamivir[b]	No treatment[c]
Nose	3	0/5*	3/4 (4.3 ± 0.1)	0/5	0/4
Lung	3	1/5 (5.5)	4/4 (3.4 ± 1.8)	0/5	0/4
Eye	6	0/5**	4/4 (2.7 ± 0.4)	0/4**	5/5 (2.9 ± 0.8)
Nose	6	1/5 (4.3)*	4/4 (3.7 ± 1.2)	0/4*	4/5 (2.3 ± 1.1)
Lung	6	2/5 (2.6 ± 1.6)**	4/4 (5.7 ± 2.2)	0/4	2/5 (1.1 ± 0.2)

[a] Values in parentheses reflect the mean viral titer in eggs ± SD of all positive samples, expressed as $\log_{10}$ EID_{50}/ml. The limit of detection is 0.8 $\log_{10}$ EID_{50}/ml, with tissues homogenized and titrated as previously described (5). *, $P < 0.05$, and **, $P < 0.005$, compared with untreated mice with homologous virus challenge. Significance was determined by Student's *t* test as previously described (5).

[b] Oseltamivir was administered once daily by oral gavage (50 mg/kg), starting 24 h preinoculation for 8 days total.

[c] Distilled water was administered by oral gavage as a control on the same schedule as oseltamivir.

vir (BioCryst) (Table 1); this assay has been shown previously to be more predictive than cell-culture-based systems for the assessment of influenza NA inhibitor drug susceptibility (12, 23, 26). Fifty percent inhibitory concentration values (IC_{50}s) of each virus were determined using a fluorescent NA inhibition assay performed as described previously (18). All H7 subtype viruses tested (covering multiple NA subtypes) were sensitive to all NA inhibitors examined, with levels of inhibitory enzyme activity comparable to those of other susceptible seasonal influenza A and B viruses (19).

To determine the sensitivity of H7 influenza viruses to oseltamivir *in vivo*, we inoculated BALB/c mice by the ocular route with two H7 viruses which exhibit divergent phenotypes in this model (5). A/Netherlands/219/2003 virus (H7N7 [NL/219]) replicates efficiently in both ocular and respiratory tract tissues without prior adaptation and causes 30% mortality following ocular inoculation. In contrast, infection of mice with A/Canada/504/2004 virus (H7N3 [Can/504]) does not cause substantial morbidity, although efficient replication in ocular tissue is detected. Oseltamivir (50 mg/kg body weight; Roche) was administered by oral gavage once daily for 8 days commencing 24 h before virus inoculation (24). Control mice (inoculated but untreated) received distilled water on the same schedule. Six-week-old BALB/c mice were inoculated with 10^6 50% egg infective doses (EID_{50}) of NL/219 or Can/504 virus by the ocular route as previously described, and 5 to 6 mice/group were monitored daily for 2 weeks for morbidity (as measured by weight loss) and mortality (5). Four to five mice from each group were sacrificed on days 3 and 6 postinfection to collect eye, nose, and lung tissue for viral titration in eggs as previously described (5).

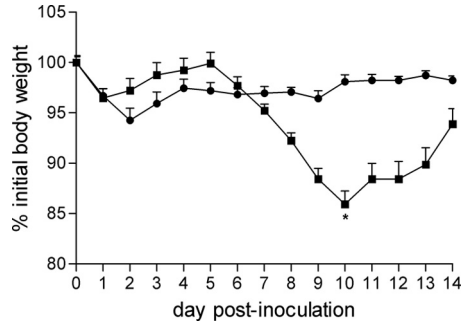

FIG 1 Efficacy of oseltamivir against NL/219 virus in mice following ocular inoculation. Oseltamivir was administered once daily by oral gavage (50 mg/kg), starting 24 h preinoculation for 8 days total. BALB/c mice were inoculated by the ocular route with 10^6 EID_{50}/5 μl NL/219 virus and monitored daily for morbidity and mortality. Data are expressed as a percentage of mean starting weight plus standard deviation. *, two mice treated with distilled water did not survive.

Oseltamivir administration was efficacious against both H7N7 and H7N3 viruses in mice, although complete inhibition of infection was not observed (Table 2). Mice inoculated by the ocular route with NL/219 virus and treated with oseltamivir were protected from weight loss and death, whereas untreated mice exhibited a mean weight loss of 14% on day 10 postinoculation (p.i.) with 30% mortality (Fig. 1). The administration of oseltamivir resulted in a decrease in both the titer and frequency of virus detection in all tissues examined in mice inoculated with H7 viruses by the ocular route. Strikingly, virus was not detected in the eyes of mice receiving oseltamivir when challenged with either NL/219 or Can/504 virus, while virus replication was detected in eyes of all untreated mice on day 6 p.i. These results suggest that oseltamivir can inhibit virus replication in ocular tissue in addition to the respiratory tract.

Isolation of virus from conjunctival swabs of persons infected with H7 viruses suggests that the clinical management of human H7 disease should include suppression of virus replication in ocular tissue. Documented spread of virus from ocular to respiratory tract tissues following ocular exposure to influenza virus in a murine model provides a further rationale for reducing viral titers in the eye via the use of antiviral drugs (J. A. Belser, P. A. Rota, and T. M. Tumpey, unpublished data). In this study, we demonstrate that both lineages of H7 subtype viruses associated with disease in humans are sensitive to existing NA inhibitors *in vitro* and that intragastric administration of oseltamivir to mice challenged with influenza virus by the ocular route inhibits virus replication in both ocular and respiratory tissues. The reduction in H7 viral titers in respiratory tract tissues is similar to that in previous studies in mice infected with an H5N1 virus and administered oseltamivir prophylaxis (27). As mice inoculated by the ocular route with influenza virus do not present with conjunctivitis (5), further study is warranted to more fully evaluate the ability of antiviral treatments to mitigate ocular disease caused by influenza virus infection. However, this is the first report demonstrating the ability of an influenza antiviral treatment to reduce viral replication in ocular tissue with viruses known to cause conjunctivitis in humans. Collectively, this study provides experimental data in sup-

port of the use of oseltamivir during outbreaks of influenza resulting in ocular disease in addition to the use of recommended personal protection (7, 8).

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