Prophylactic efficacy of AVI-7100 against Influenza A in mouse and ferret infection models

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Purpose

The objective of these studies was to evaluate the prophylactic therapeutic utility of AVI-7100 for influenza A.

Conclusions

- AVI-7100 is effective against influenza A (H1N1 and H3N2) in both mouse and ferrets when administered prior to viral challenge.
- Chemoprophylaxis with AVI-7100 is effective and the effects are long lasting.
- The respiratory elimination of AVI-7100 is zero order which corresponds to the duration of prophylactic protection .

Abstract

AVI-7100 is a phosphorodiamidate morpholino oligomer containing three modified linkages (PMO*plus*) that is designed to interfere with expression of the M1 and M2 genes of influenza A virus.

Methods. A single 0.1 mg intranasal (i.n.) dose of AVI-7100 was administered to female BALB/c mice (n=10/group) at 7 days, 3 days or 4 hours prior to viral challenge with 5 X 10^5 pfu of A/Port Chalmers/1/73 (H3N2). Lung viral load was determined on day 6 post infection. In a separate study, a single i.n. dose was administered to mice infected with A/PR/8 (H1N1) and plasma and lung oligomer concentrations were determined. Outbred ferrets (*Mustela putorius furo*; n=7/group) were administered AVI-7100 as a single i.n. dose at 7 days, 5 days, 3 days or 4 hours prior to insufflation viral challenge with 5 X 10^5 pfu H1N1 A/Hong Kong/2369/09 per ferret. Negative control groups were treated with saline and positive controls were administered oseltamivir at 10 mg/kg p.o. every other day beginning 7 days prior to infection.

Results. A single intranasal dose of AVI-7100 (0.1 mg/mouse) administered 7 days, 3 days or 4 hours prior to infection with A/Port Chalmers/1/73 (H3N2) significantly ($p<0.05$) reduced lung viral titers in each group compared to vehicle controls and oseltamivir treated mice. PMO*plus* concentrations in the lungs of mice following a single insufflation dose follow zero order elimination and tissue concentrations above the AVI-7100 EC50 are maintained for greater than three days. In the ferret, a single i.n. dose of AVI-7100 administered 7 Days, 5 days, 3 days or 4 hours prior to exposure with A/Hong Kong/2369/09 (an oseltamivir resistant pH1N1) significantly ($p<0.05$) reduced cumulative viral load in nasal wash and in lung bronchiolar lavage compared to saline controls and oseltamivir treated ferrets. The decrease in viral load in nasal wash samples was directly proportional to the interval of time between prophylactic treatment and viral exposure (a zero order reduction in activity).

Conclusions. AVI-7100 is effective against influenza A (H1N1 and H3N2) and in both mouse and ferrets after a single intranasal dose for up to seven days prior to viral exposure. Zero order elimination of AVI-7100 from the lung was observed. These observations support the prophylactic use of AVI-7100 in preventing influenza A infection.

Introduction

The 2009 pandemic influenza (H1N1) virus spread worldwide despite attempts at containment including isolation, quarantine and screening. Vaccines proved to be of limited utility resulting in incomplete global protection. Chemo-prophylaxis with oseltamivir reduced the number of cases attributable to the index case from 1.91 before chemoprophylaxis to 0.11 after chemoprophylaxis (Lee et al., 2010). Chemoprophylaxis for household transmission of influenza and may represent a "ring prophylaxis" strategy for upcoming pandemics.

Post-exposure prophylaxis with oseltamivir was linked to emergence of an H275Y mutation in the neuraminidase protein. This mutation resulted in an increase in the 50% inhibitory concentration of oseltamivir from 0.27 nM to >400 nM but the virus remained susceptible to zanamivir (Baz et al., 2009). The H275Y mutation is also observed in seasonal H1N1 and avian H5N1 strains (Dharan et al., 2009; deJong eg al., 2005). The fact that resistance is a point mutation away may limit the utility of neuraminidase inhibitors for chemoprophylaxis.

An urgent need exists for new forms of treatment for influenza A based on (a) the known propensity of this virus to undergo both continuous low-level antigenic drift and less frequent but unpredictable major antigenic shift leading to pandemic disease, (b) the clear failure of vaccination, even when strains are reasonably matched, to prevent influenza-related illness in a significant proportion of vaccine recipients, and (c) the increased frequency of resistance to approved forms of therapy for influenza (e.g., the adamantane derivatives and, more recently, the neuraminidase inhibitor, oseltamivir).

Earlier work with the peptide conjugated phosphorodiamidate morpholino oligomer (PPMO) identified effective inhibitors of viral titer with doses as low as 0.15mg/kg resulting in 1.9 to 2.0 log reduction in lung viral titer and 50 percent survival in a lethal challenge mouse model (Ge et al., 2006; Gabriel et al., 2008; Lupfer et al., 2008).

In 2009, a rapid response project to identify an RNA based therapeutic for pandemic H1N1 was initiated. Funding from the U.S. Department of Defense supported the rapid identification, design, and manufacture of therapeutic candidates in less than two weeks, preclinical development of AVI-7100, an Investigational New Drug (IND) application with the U.S. Food and Drug Administration, and initiation of a Phase 1 clinical trial to obtain human safety data. AVI-7100, a PMO*plus* oligomer, is specifically designed to interfere with a conserved target across various influenza A strains targeting the expression of the M1 and M2 genes.

Literature Cited:

Baz M, Abed Y, Papenburg J, Bouhy X, Hamelin M and Boivin G (2009) Emergence of Oseltamivir-Resistant Pandemic H1N1 Virus during Prophylaxis. *NEngl J Med* **361**:2296-2297.

Dharan NJ Gubareva N, Biovin GA et al. (2009) Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* **301**:1034-1041.

De Jong MD, Tran TT, Truong HK et al. (2005) Oseltamivir resistance during treatment of influenza A (H5N1) infection. *NEngl J Med* **353**:2667-2672.

Ge Q, Pastey M, Kobasa D, Puthavathana P, Lupfer C, Bestwick RK, Iversen PL, Chen J and Stein DA (2006) Inhibition of multiple subtypes of influenza a virus in cell cultures with morpholino oligomers. *Antimicrob Agents Chemother.* **50(11)**:3724-3733.

Gabriel G, Nordman A, Stein DA, Iversen PL and Klend H-D (2008) Morpholino Oligomers Targeting the PB1 and NP Genes Enhance Survival of Mice Infected with highly Pathogenic Influenza A H7N7 Virus. *J Gen Virology* **89**: 939-948.

Lee VJ, Yap J, Cook AR, Chen MI, Tay JK, Tan BH, Loh JP, Chew SW, Koh WH, Lin R, Cui L, Lee CWH, Sung WS, Wong CW, Hibberd ML, Kang WL, Med M, Seet N and Tambyah PA (2010) Oseltamivir Ring Prophylaxis for Containment of 2009 H1N1 Influenza Outbreaks. *NEngl J Med* **362**:2166-2174.

Lupfer C, Stein DA, Mourich DV, Tepper SE, Iversen PL and Pastey M (2008) Inhibition of influenza A H3N8 virus infections in mice by morpholino oligomers. *Arch Virol.* **153**: 929-937.

Support

This study was conducted under contract with the Department of Defense Joint Project Manager Transformational Medical Technologies.

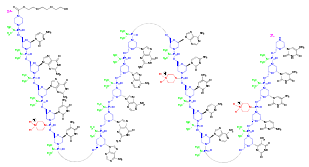
Background

AVI-7100 Targets a Highly Conserved Viral Sequence in Influenza A (subscripts indicate percent conservation at that sequence position):

5'-$A_{99.6}A_{100}A_{100}G_{99.6}A_{99.7}T_{99.9}G_{99.9}A_{99.9}G_{99.9}T_{99.9}C_{99.9}T_{99.}{}_9T_{99.9}C_{100}T_{100}A_{99.}{}_9A_{100}C_{100}C_{100}G_{100}$-3'

NCBI Influenza Virus Resource (11/10/2009)
(database utilized for sequence analysis):
H1N1 – 845 seqs from humans
H1N1 (S-OIV) – 775 seqs from humans
H5N1 – 947 seqs from all species
H3N2 – 835 from humans in North America
H9N2 – 348seqs from all species
H2N2 – 107seqs from all species
H7N7 – 35seqs from all species

AVI-7100

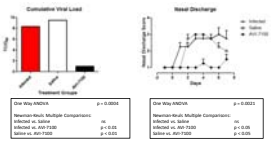


AVI-7100 Efficacy Studies Summary:

1. Effective in mouse and ferret after single i.n. administration.
2. Effective in mouse and ferret after daily (4-6) i.p. doses .
3. Effective against neuraminidase resistant pH1N1 (A/Hong Kong/2369/09) in ferret model.
4. Effective in immune suppressed (CD4 depleted) and autoimmune (EAE) mouse models.
5. Effective in blocking viral transmission (see figure below)

AVI-7100 Prevents Transmission of Oseltamivir Resistant pH1N1 from Infected to Naive Ferrets

Group	Treatment	Dose	Route	Schedule	Number
1	AVI-7100	3 mg/kg	i.n.	-1D, 0D, 1D, 2D, 3D, 4D, 5D	6; 2 infected, 4 naive
2	Saline	-	i.n.	-1D, 0D, 1D, 2D, 3D, 4D, 5D	6; 2 infected, 4 naive



One Way ANOVA	p < 0.0001
Newman-Keuls Multiple Comparisons:	
Infected vs. Saline	ns
Infected vs. AVI-7100	p < 0.01
Saline vs. AVI-7100	p < 0.01

One Way ANOVA	p = 0.0021
Newman-Keuls Multiple Comparisons:	
Infected vs. Saline	ns
Infected vs. AVI-7100	p < 0.05
Saline vs. AVI-7100	p < 0.05

Prophylactic Efficacy

Mouse

Table 1. Study Design for Balb/c Female Mice infected with A/Port Chalmers/1/73 (H3N2) via the intranasal route

Group	Treatment	Number	Dose	Route	Schedule
1	Saline	10	-	i.n.	-7D, -3D, -4H
2	Oseltamivir	10	10 mg/kg/dose[1]	p.o.	-7D, -3D, -4H
3	AVI-7100	10	5 mg/kg[2]	i.n.	-7D
4	AVI-7100	10	5 mg/kg	i.n.	-3D
5	AVI-7100	10	5 mg/kg	i.n.	-4H
6	AVI-7100	10	5 mg/kg/dose	i.n.	-7D, -4H
7	AVI-7100	10	5 mg/kg/dose	i.n.	-7D, -3D, -4H
8	Scramble	10	5 mg/kg/dose	i.n.	-7D, -3D, -4H

[1] dose volume 200 µl/dose
[2] dose volume 50 µl/dose

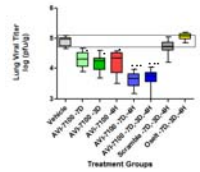


Figure 1: AVI-7100 reduced lung viral titer in female Balb/c mice when administered 7D, 3D, and 4H prior to infection (-7D, -3D, and -4H) or 7D + 3D +4H prior to infection (-7D, -3D, -4H). Single asterisk indicates $p < 0.05$ versus oseltamivir and double asterisk indicates $p<0.05$ versus saline.

Ferret

Table 2. Study Design for male *Mustela putorius furo* infected with A/Hong Kong/2369/09(H1N1) via the intranasal route[1]

Group	Treatment	Number	Dose	Route	Schedule
1	Saline	7	-	i.n.	-7D, -5D, -3D, -1D
2	Oseltamivir	7	10 mg/kg[2]	p.o.	-7D, -5D, -3D, -1D
3	AVI-7100	7	3 mg/kg[3]	i.n.	-7 Day
4	AVI-7100	7	3 mg/kg	i.n.	-5 Day
5	AVI-7100	7	3 mg/kg	i.n.	-3 Day
6	AVI-7100	7	3 mg/kg	i.n.	-1 Day

[1] 5 X 10^5 pfu/ferret in 200 µl
[2] dose volume 200 µl/dose
[3] dose volume 200 µl/dose

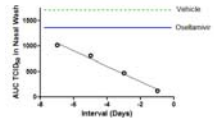


Figure 2: AVI-7100 reduced nasal wash viral titer in male ferrets when administered 7, 5, 3, and 1 day prior to infection (-7, -5, -3, and -1D) infection. Blue line represents the oseltamivir group and the dashed line represents the vehicle control group. A linear regression fit to these data result in a line with significant slope ($p = 0.0059$; $r2 = 0.9883$) and indicate a reduction in the AUC TCID50/mL of 150 per day.

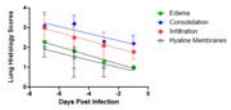


Figure 3: Microscopic evaluation of the lung. Scores are: 1 = Normal, 2 = Mild Changes, 3 = Moderate Changes, and 4 = Severe Changes. Samples were blinded to the investigator. Edema, infiltration and hyaline membrane scores all have significant slopes ($r^2 = 0.987$, $p = 0.0065$; $r^2 = 0.989$, $p = 0.0055$; and $r^2 = 0.916$, $p = 0.0427$, respectively).

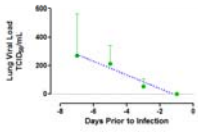


Figure 4: AVI-7100 reduced lung viral titer in male ferrets when administered 7, 5, 3, and 1 day prior to infection (-7, -5, -3, and -1D).

Lung Residence Time Correlates Well with Duration of Prophylactic Efficacy

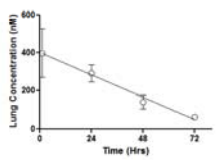


Figure 5: Lung concentrations of PMO*plus* versus elapsed time following intranasal dose to mice. Concentrations were determined from uninfected mice at various times after intranasal administration. Elimination profiles reveal a zero order clearance at approximately 4.8 ± 1.2 nM/hr (slope significantly different from 0 ($p = 0.0014$).

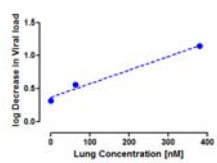


Figure 6: Viral load observations from prophylactic treatment regimens in Figure 1 are plotted on the Y-axis against observed lung concentrations of PMOplus on the X-axis. The $r^2 = 0.98 \pm 0.08$ with slope of 0.002 ± 0.0003 change in viral load per nM PMO*plus* ($p = 0.089$).